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ON THE MORPHOLOGY AND LIFE HISTORY OF *APROCTONEMA ENTOMOPHAGUM* KEILIN, A NEMATODE PARASITE IN THE LARVAE OF *SCIARA PULLULA* WINN. (DIPTERA—NEMATOCERA).

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(With Plates XIX and XX, containing Figs. 1–19, and Figs. I, II in the Text[1].)

INTRODUCTION.

APROCTONEMA ENTOMOPHAGUM was found at Cambridge by one of us (D. K.) during the examination of the larvae of a Mycetophilid fly, *Sciara pullula* Winn., inhabiting the decaying wood of an elm. When the larvae were placed in water under a binocular microscope, many of them were seen to differ conspicuously from their fellows in being filled with one or more large, milk-white bodies. These proved to be female Nematodes, occupying the body cavity of their hosts; closer examination showed that they were accompanied by minute males. A short note on the parasite was published by Keilin in 1917. We propose now to give a more complete account of the anatomy, biology and systematic position of this parasite.

ANATOMY.

Mature female (Fig. II): 4·4–6 mm. long, 0·22–0·30 mm. broad. The distance from the vulva to the posterior end is 1·7 mm. The worm is elongated, with both ends rounded, white or slightly greenish in colour and very soft and fragile. The cuticle is extremely thin and has longitudinal and transverse, but no diagonal fibres. There are no lateral organs and no cuticular expansions or papillae.

The mouth is minute, without lips and bears a small piercing stylet; the oesophagus is more or less straight and the lumen is minute, but traceable as far as the intestine when it becomes lost. The intestine is composed of very large cells surrounding a central strand (Fig. I, *2*) and occupies nearly the whole breadth of the body cavity, lying dorsal to the genital organs; at its junction with the oesophagus it gives off a solid diverticulum which runs forwards, dorsal to the oesophagus, for about half its length. The intestine ends blindly near the hind end of the body; there is no anus.

There are two opposed, non-reflexed ovaries, the posterior one starting

[1] Note that *Plate* figures are cited in *Arabic* numbers and *Text* figures in *Roman* numbers throughout. Ed.

near the hind end of the body and the anterior one at the front end of the intestinal diverticulum. Each ovary becomes an oviduct which widens into a uterus. The two uteri converge to the vagina, which is a short tube running

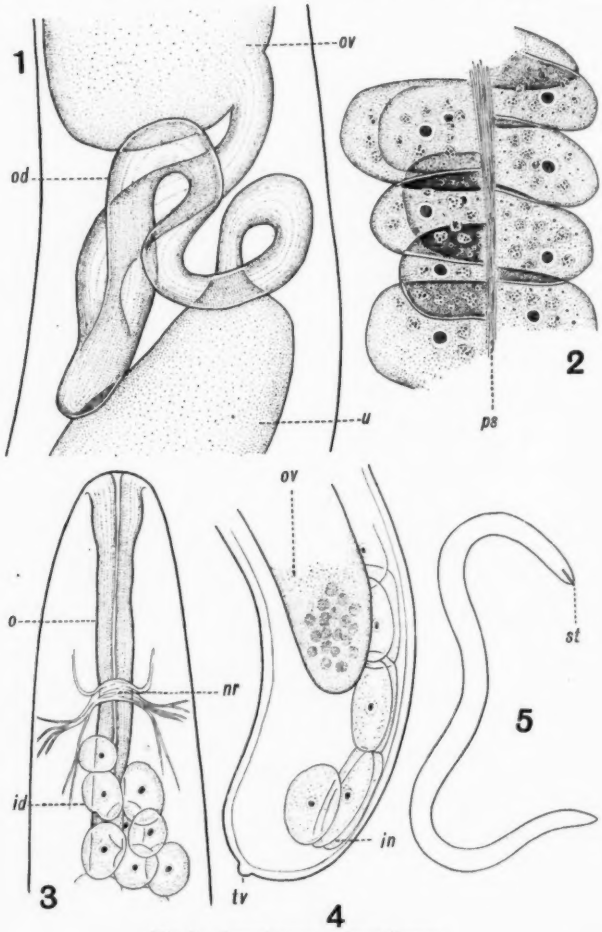

Fig. I. *Aproctonema entomophagum.*

1. Portion of genital tube of the immature ♀ from the Mycetophilid larva shown in (Plate) Fig. 2 (partly schematised). ×420.
2. Optical section of portion of intestine of an adult ♀. ×210.
3. Head end of the same ♀ as (1). ×210.
4. Posterior end of the same ♀ as (1), showing abnormal terminal vesicle. ×210.
5. Outline drawing of newly hatched larva. ×210.

at right angles to the long axis of the body and opening at the vulva, situated just behind the middle of the body and projecting but slightly from the surface. The animal is oviparous, and the eggs, which are spherical, are

0·09 mm. in diameter and provided with a very thin, unornamented egg-shell.

There are three large rounded cells (possibly the excretory cells), two situated at the front end of the anterior ovary, the third immediately in front of the vulva; this one appears to migrate backwards during development, since in young females it lies just behind the oesophagus (Fig. 12). No excretory pore could be seen in either sex.

The nerve-ring lies across the oesophagus at a distance of 0·13 mm. from the anterior end.

Male. The mature male before copulation is 1·9 mm. long and 0·1 mm. broad. Both extremities are rounded, but the tail end bears a small, terminal, transparent knob or vesicle. There are no cuticular expansions or lateral organs.

The alimentary canal and the stylet are the same as in the female, except that the oesophagus is thrown into a loop, like that of the larvae of both sexes (see *Development*).

The testis comprises two opposed, non-reflexed portions, the posterior starting near the hind end of the body, the anterior at the level of the oesophageal loop. The two portions meet at a point a little way behind the middle of the body to form a vas deferens or ejaculatory duct, which runs backwards, ventral to the posterior portion of the testis, to open into the bulb of the spicule. The two portions of the testis are different in appearance and probably in function also; the posterior portion in life continually undergoes peristaltic movements and may perhaps be regarded as a seminal vesicle, but for systematic purposes the testis must be said to be opposed. The single, median spicule is 0·09 mm. long, 0·01 mm. broad at its proximal end and 0·004 mm. at its distal end; it is slightly curved and lies in a sheath or bulb provided with spicular muscle fibres. There is no gubernaculum.

The male after copulation (Fig. 8) shrinks to a small, wrinkled body (0·26 × 0·07 mm.), in which none of the organs are recognisable, except the spicule.

Larvae (Fig. 10). In both sexes the oesophagus is at first straight and measures more than half the length of the body. The intestine ends blindly from the beginning and there is no double oesophageal bulb. The piercing stylet, which is present in both sexes, measures 0·016 × 0·002 mm. (Fig. 9).

Development (Figs. 10–13, 15 and 16). The oesophagus grows in length more rapidly than the body of the worm and thus early comes to form a loop on itself. From the front end of the intestine the intestinal diverticulum grows forwards; it has the same central strand as the rest of the intestine and is multicellular.

In both sexes the genital rudiment appears in the same place, somewhat behind the middle of the body. In the female the vulva soon becomes evident on the surface opposite the centre of the rudiment; from the corresponding point in the male the ejaculatory duct grows backwards towards the developing spicule. The genital rudiment extends forwards and backwards and

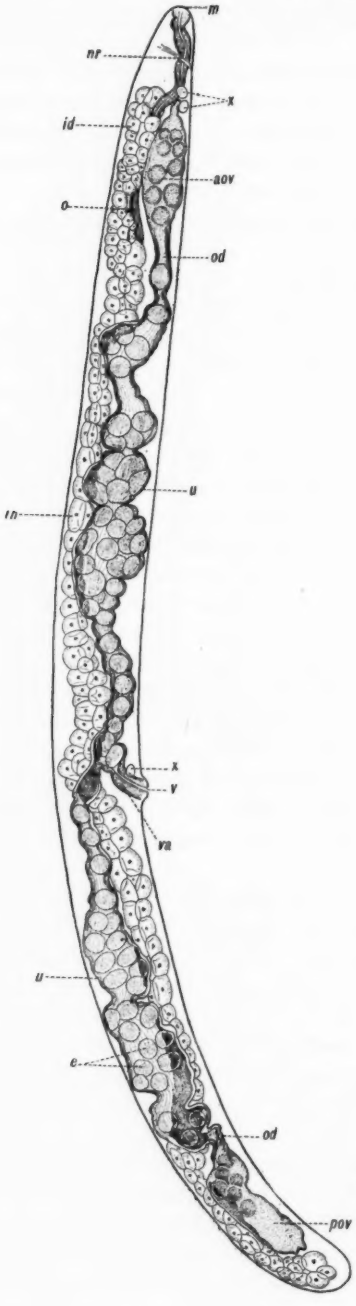

Fig. II.
Aproctonema entomophagum.
Mature female. ×47.

in the female each branch becomes differentiated into a genital tube and an ovary (Fig. 12). At a somewhat later stage (Fig. 13) each genital tube is seen to consist of a capacious uterus, springing directly from the vagina, and a narrower oviduct, connecting the uterus with the ovary. At first the oviduct is straight, but some time after copulation it becomes complexly coiled (Fig. I, *1*). As the worm grows in size, the entire genital tube from vagina to ovary becomes packed with fertilised eggs and the whole body is greatly distended, so that not only the coils of the oviduct, but also the oesophageal loop, are straightened out.

Abnormalities. On two occasions abnormalities in the shape of the posterior end were observed. Fig. I, *4* shows part of a growing female with a terminal vesicle resembling that borne normally by the male. Fig. 8 represents two shrunken males, in one of which the tail is produced into an elongated spike.

BIOLOGY.

The host larvae are so transparent that the entire biology of the parasites, including copulation and the various processes of growth and degeneration, can be watched *in vivo* within them. Thus all the parasitic stages can be followed directly by observing a given host from time to time.

A single mature female worm is so large that it occupies the greater part of the body cavity of its host (Fig. 1). The majority of larvae, however, contain more than one female together with a variable number of small males. Fig. 6 shows a host larva literally stuffed with nearly mature Nematodes. As many as twelve parasites (ten males and two large females) have been found in one host. Various stages of the infection are shown in Figs. 1–6.

Recently infected larvae contain worms which are all more or less equal in size. At this stage copulation (observed only on one occasion) takes place. Shortly afterwards the male starts to shrink, its cuticle becomes wrinkled and eventually it is reduced to a short, stumpy worm about 1/20th the length of the mature female. It then dies, while the female, at first about 1·9 mm. long (Fig. 13), with numerous spermatozoa in the genital tubes, grows enormously, the uteri becoming packed with fertilised eggs.

The adult female worm now escapes from the insect larva and expels its eggs. The dead bodies of Mycetophilid larvae were frequently found between wood-fibres lying beside the remains of the female Nematode covered with eggs. The actual escape has been observed once only: a Mycetophilid larva was found with a tear near its anterior end, from which protruded the female worm together with some of the viscera of the larva.

Once free from its host the worm becomes quite active, curling and uncurling itself while the eggs are laid. The head and tail ends are much more active than the middle region and their movements remind one forcibly of those of an aquatic Oligochaete. At this stage the extreme thinness of the cuticle can be appreciated; on being lifted or moved the worm behaves exactly like a small bag of jelly, collapsing limply over the needle.

The eggs are not expelled by bursting the parent, but are delivered through the vagina one by one. They are distorted in hour-glass fashion by their passage through the vulva, but soon regain their spherical shape. The number of eggs laid by one female is not great, 216 being a count in what was probably a usual case. After expelling them the female dies. The extrusion of the polar bodies occurs shortly after the eggs are laid and segmentation then begins. After a few days the egg is found to contain a young larva, which is coiled in a spiral inside the egg-shell (Fig. 18) and bears an obvious buccal stylet, usually protruded on hatching. The newly hatched larvae, packed with minute granules, measure 0·46 × 0·017 mm. and are reminiscent of young Mermithids (Fig. I, *5*). They begin to moult actually before hatching and the first larval skin with its buccal stylet is left in the egg-shell. This was the only ecdysis observed throughout the life cycle.

The youngest worms seen in the body cavity of the host have the same structure and dimensions as the newly hatched larvae, and there seems therefore no doubt that their free-living stage is a short one. Their actual penetration into the host has never been observed, but the Mycetophilid larvae which contained the young parasites bore scars on their skin, which were probably the portals of entry (Fig. 5). The mandibles of the host probably prevent the entrance of the larvae through the mouth; in the closely related form, *Tetradonema plicans*, Hungerford (1919) states that the eggs of the parasite are probably swallowed, since he found very young larvae in the gut of the host. Nothing of the kind has been seen in the case of *Aproctonema*.

If parasitism occurs late in the life of the host, the parasites are carried over through the pupal stage of the host into the imago. All stages of the insect have been found parasitised.

The escape of the worm from the adult fly does not take place in the same way as from the larvae. The mature female *Sciara*, during oviposition, inserts the posterior end of her abdomen between the cracks of the decaying wood and deposits a packet of eggs. Some of these female flies were found, in which the abdomen was much elongated and occupied by a mature female Nematode (Fig. 7). The worm had stretched the abdomen to such an extent that the last few sclerites were separated from one another by wide intervals of thin, intersegmental chitin, forming a long hernia, whose apex bearing the ovipositor suddenly burst, liberating the worm. During this process the fly made the normal convulsive movements of egg-laying. After the escape of the worm the fly died, while the Nematode began to expel its eggs.

It is possible that infection of the adult flies also occurs by direct penetration by the young Nematode larvae, as happens in Mermithids, since the adult flies run about the wood-fibres in company with the young stages and some of them have been found containing two generations of worms, one being obviously quite recent.

Effect on the host.

Larva. In one batch of host insects a number of parasitised larvae were found together with some mature flies, none of which were parasitised. All these stages belonged to the same generation, and it is therefore probable that parasitism delays the metamorphosis of the host. It is perhaps owing to this delay that some larvae were found containing at least two generations of Nematodes—one consisting of mature females and degenerated adult males, the other of young larvae.

Imago. It is remarkable that the only parasitised imagines found were females. Sections of such insects were rather unsatisfactory, but they showed that the abdominal organs of the fly were considerably compressed by the large bulk of the parasite and no ovaries were visible; nevertheless, as has been described, the internal pressure caused by the parasite provokes the normal egg-laying response in the fly. Lack of fresh material makes it impossible to say whether the males are actually parasitised, but die off early, or whether the parasitism of the larval stages brings about some change leading to the production of the female sex only. The other Nematode, *Tetradonema plicans*, also found in *Sciara*, appears to parasitise the imagines of both sexes, but such flies are sterile (Hungerford, 1919). The method of escape of this parasite from the host was not described.

SYSTEMATICS.

It is evident that *Aproctonema* must occupy a systematic position somewhere among the following aberrant Nematodes: *Allantonema* Leuckart 1884, *Bradynema* zur Strassen 1892, *Howardula* Cobb 1921, *Sphaerularia* Dufour 1837, *Atractonema* Leuckart 1887, *Tylenchinema* Goodey 1930, *Myenchus* Schuberg and Schröder 1904, *Myoryctes* Eberth 1863, *Mermis* Dujardin 1842, and *Tetradonema* Cobb 1919.

The first eight of these have been grouped together in one family, the Myenchidae, by Pereira (1931).

Aproctonema differs from them in showing the following characters:

(1) The genital tubes are double and opposed in both sexes, each branch bearing an ovary or testis. The other genera have single anterior genital tubes and gonads.

(2) There is only one spicule, all the others having two.

(3) There are no caudal alae in the male.

(4) The anus is absent throughout life in both sexes. It is present, though vestigial, in the free-living forms of both sexes in *Allantonema* and *Bradynema*, present in the male only in *Sphaerularia* and *Atractonema*, present throughout life in both sexes of *Tylenchinema* and vestigial in *Howardula*, *Myenchus* and *Myoryctes*.

(5) The free-living stage is much shorter than in any of the other forms (except *Myenchus* and possibly *Myoryctes*).

In the first three characters and also in the general shape of the larvae *Aproctonema* closely resembles many of the Mermithidae. In fact Baylis and Daubney (1926) make both *Aproctonema* and *Tetradonema* synonymous with *Mermis*. The last differs however from the other two in the following characters:

(1) The presence of cephalic, circumoral and caudal papillae.
(2) The presence of lateral organs.
(3) The presence of an anus in the larva.
(4) The presence of a buccal stylet only in the larval stages.
(5) The much shorter parasitic life.

It is evident that if one generic definition is to cover all these three forms, a large number of other genera of Nematoda will have to fall into synonymy.

The nearest relative of *Aproctonema* is the American form, *Tetradonema plicans* Cobb 1919, which has a similar life history in *Sciara coprophila* Lintner. Cobb created a new family, the Tetradonematidae, with *Tetradonema* as the type-genus, having the following definition:

"Small, naked insect parasites with minute males; cuticula wingless, minutely transversely striated; head rounded, tail acute; mouth minute, lipless, oral papillae minute, anus none; oesophagus simple, with lumen, intestine vestigial; male and female gonads double, symmetrically reflexed; vulva central, uterine eggs numerous, asymmetrical, containing embryos; spiculum without accessories, supplements and bursa none."

This definition covers *Aproctonema* also, with the exception of certain differences, which serve, temporarily at any rate, to keep the two forms in separate genera. These are:

	Tetradonema.	*Aproctonema.*
(1)	Minute circumoral papillae.	None.
(2)	Tail acute in both sexes.	Tail rounded in both sexes, but bearing a small terminal vesicle in the male.
(3)	Dorsal to the oesophagus there is a peculiar organ, unconnected with the alimentary canal, consisting of a longitudinal row of four very large cells and named by Cobb the "tetrad."	The position of the "tetrad" is occupied by the intestinal diverticulum.
(4)	Intestine not traceable further back than the middle of the body.	Extends almost to hind end.
(5)	Both ovaries and both testes reflexed.	Not reflexed.
(6)	The male is 0·8 mm. long.	1·9 mm. long.
(7)	Eggs shaped like mushroom caps.	Spherical.

No mention is made by Cobb of a stylet in either sex of *Tetradonema*, but neither does he describe larvae and presumably was working with preserved material, in which (at any rate in the adult *Aproctonema*) it is impossible to make out a stylet even when its presence is known from an examination of the living worm. In view of this inadequacy of description and the close relationship of both forms to *Mermis*, we regard *Aproctonema*, *Tetradonema* and *Mermis* as three genera of the family Mermithidae. On the other hand, if the family Tetradonematidae is allowed to stand, its name must be changed to Aproctonematidae, since the first account of *Aproctonema* was published two years before *Tetradonema* was described and named.

The peculiar intestinal diverticulum in *Aproctonema* is perhaps homologous with the "tetrad" of *Tetradonema*, and may be, either, as Cobb suggests, a storage place for waste products in an animal which spends most of its life as an internal parasite and has no excretory pore, or it may serve as an extra food store. There is no sign in these two forms of the unicellular "intestinal gland" found in *Allantonema*, *Bradynema* and *Tylenchinema*, unless it is represented by the peculiar intestinal diverticulum just described. In this connection it is perhaps noteworthy that the two structures occupy corresponding positions and that in *Allantonema* and *Bradynema* the "intestinal gland" has no duct.

DISCUSSION.

The life cycle of *Aproctonema* (and *Tetradonema*) differs from that of the other Nematodes parasitic in insects in the two following particulars: first, the very short free-living stage, and, second, the fact that the males are parasitic as well as the females. Sexual dimorphism, though striking in the very late stages, when the females are distended with eggs and the males are shrunken, does not exist in the younger individuals, and is actually no more marked than in such forms as *Allantonema* or *Sphaerularia*.

Statements are frequent in the literature that protandrous hermaphroditism occurs in many Nematodes parasitic in insects. The phenomenon was recorded for *Allantonema mirabile* by Leuckart (1887), for *Bradynema rigidum* by zur Strassen (1892) and for *Howardula benigna* by Cobb (1921). In addition Pereira (1931) claims that the allied form, *Myenchus botelhoi*, a parasite of leeches, exhibits protandry. Wülker (1923), however, in a re-investigation of the subject, concluded that *Allantonema*, *Bradynema*, *Tylenchus* and *Howardula* all have similar life histories: the host is penetrated by a female, which has been spermatised while in the free-living stage. Thus the individuals regarded by the older observers as protandrous hermaphrodites were simply spermatised females. The same thing occurs in *Tylenchinema* and probably also in *Mermis*, while the case of *Myenchus* requires confirmation.

In two of these forms at least (*Allantonema mirabile* and *Tylenchinema oscinellae*) spermatisation occurs very early, while the ovary is still composed of a few cells only. In *Aproctonema* copulation does not take place until the ovary is fully developed, but the appearance of the spermatised females, containing both ova and sperm, would certainly be misleading, if the males were not present in the same host. The study of the life history of *Aproctonema* revealed no evidence for protandrous hermaphroditism.

SUMMARY.

1. An account is given of *Aproctonema entomophagum* Keilin 1917, a Nematode parasite of the larvae of a Mycetophilid fly, *Sciara pullula* Winn., inhabiting decaying wood.

2. The life history of this parasite is remarkable in the following respects:

(*a*) The free-living stage is very short.

(*b*) Both sexes are parasitic.

(*c*) The usual host is the larva of the fly, from which the parasite escapes by actively breaking through the skin; but some parasites may be carried through to the imagines, from which they escape largely by the aid of the egg-laying movements provoked by them in the flies.

3. Metamorphosis of the *Sciara* larva is delayed by the parasitism, so that at least two generations of Nematodes may be found in one host.

4. All the parasitised imagines so far examined have been females.

5. The systematic position of *Aproctonema* is discussed, the conclusion being that this worm and its near relative, *Tetradonema plicans* Cobb 1919, represent two closely allied genera of the family Mermithidae, but are not synonymous with *Mermis*. It is proposed to dispense with the family Tetradonematidae Cobb.

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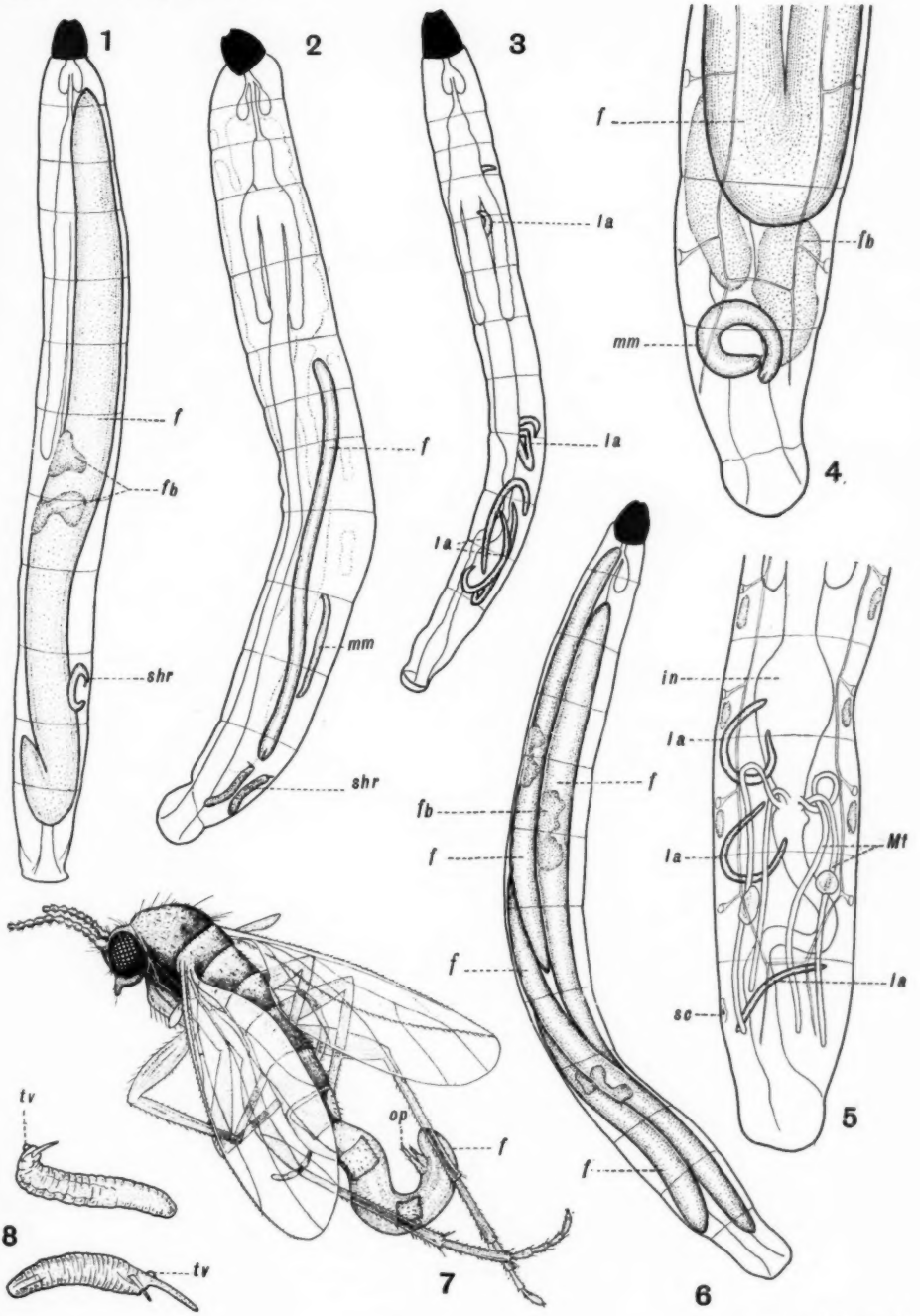
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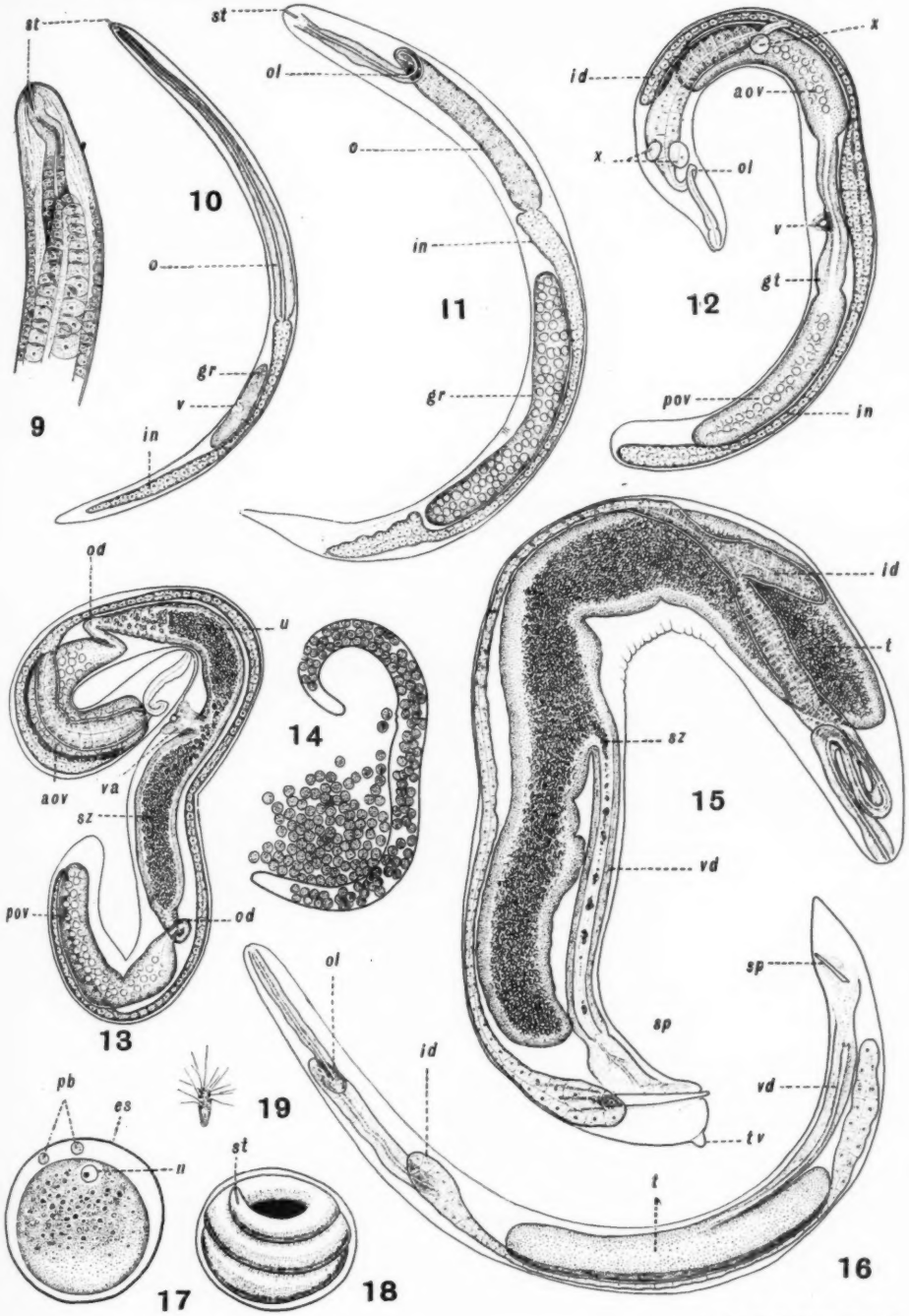
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EXPLANATION OF PLATES XIX AND XX.

Illustrating the nematode *Aproctonema entomophagum*, parasitic in larvae of the nematocerous insect *Sciara pullula*.

PLATE XIX.

Fig. 1. Larva of *Sciara*, containing one fully grown ♀ and one shrunken ♂. ×22.
Fig. 2. Larva of *Sciara*, containing one growing ♀, one large ♂ and two shrunken ♂♂. ×22.
Fig. 3. Larva of *Sciara*, containing five larval Nematodes. ×22.
Fig. 4. Larva of *Sciara*, posterior end, showing one small ♂ and part of a fully grown ♀. ×50.
Fig. 5. Larva of *Sciara*, posterior end, showing three larval Nematodes and a scar on the skin of the host. ×50.
Fig. 6. Larva of *Sciara*, containing four large ♀♀. ×22.
Fig. 7. Imago of *Sciara*, showing escape of large ♀ Nematode; preserved specimen. ×30.
Fig. 8. Two shrunken ♂♂, one with an abnormal tail end. ×80.

PLATE XX.

Fig. 9. Head end of larval Nematode, showing stylet; preserved specimen. ×300.
Fig. 10. Very young female larva; compressed. ×200.
Fig. 11. Young female larva; compressed. ×200.
Fig. 12. Older female larva; compressed. ×80.
Fig. 13. Spermatised ♀; compressed. ×80.
Fig. 14. Fully grown ♀, expelling eggs. ×22.
Fig. 15. Mature ♂, after copulation; compressed. ×200.
Fig. 16. Young male larva; compressed. ×200.
Fig. 17. Fertilised egg. ×280.
Fig. 18. Young larva before hatching. ×280.
Fig. 19. Spermatozoon. ×466.

KEY TO LETTERING OF PLATES AND TEXT-FIGURES.

aov anterior ovary
e egg
es egg-shell
f female
fb fat-body
gr genital rudiment
gt genital tube
id intestinal diverticulum
in intestine
la larva
m mouth
mm male
Mt Malpighian tubule (host)
n nucleus
nr nerve-ring
o oesophagus
od oviduct
ol oesophageal loop
op ovipositor (host)
ov ovary
pb polar body
pov posterior ovary
ps pseudo-lumen
sc scar (host)
shr shrunken male
sp spicule
st stylet
sz spermatozoa
t testis
tv terminal vesicle
u uterus
v vulva
va vagina
vd vas deferens
x excretory cell?

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ON THE BIOLOGY AND LIFE HISTORY OF *RHABDITIS PELLIO* (NEMATODA).

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(With 1 Graph.)

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I. Introduction.

Rhabditis pellio occurs in the living earthworm only in two larval stages, one encysted in the coelom, and the other living free within the bladder of the nephridia. Sexually mature males and females never occur within the living earthworm, but if the earthworm dies, the larvae from the coelom and nephridia become mature on its decaying body, the offspring from these passing through the soil to infect other earthworms. Johnson (1913), who worked on this species, summarises all the literature up to 1913, and gives full descriptions and illustrations of the adults and larvae.

The larvae inhabiting the coelom, according to Johnson, occur most commonly encysted among the "brown bodies" in the posterior segments of the earthworm, to which region they are worked by the movements of its body. A few encysted larvae sometimes occur embedded in the muscular layer of the body wall, or encysted upon the septa, and occasionally a few unencysted larvae are found free in the coelom. Johnson describes the cyst as being usually in the shape of a 3 or 8, and slightly longer than the nematode,

thus allowing it to move backward and forward. On emerging from the cyst the larva pushes off the anterior end.

The active nephridial larva was only found by Johnson in the bladder of the nephridia, but Lankester (1865) found it also in the seminal vesicles. This nephridial form, although the more numerous, appears to have been neglected by most of the early workers, and up to 1913 it was not known for certain whether these two larval stages belonged to the same species. Johnson, however, by rearing both the encysted and nephridial larvae to maturity, found that they were both larval stages of one and the same species.

R. pellio does not appear to harm the host earthworm in any way, for healthy earthworms may be very heavily infected.

II. Systematic.

A certain confusion appears to have existed over the naming of this species, with the result, that a free-living soil species of *Rhabditis* has received the same name as this parasite. Bütschli, writing in 1873, noticed a certain difference in the tails of his male specimens, obtained from a decaying earthworm, and those described by Schneider, who first named the adults in 1866. Most of the other workers, however, appeared to have overlooked these small differences until Maupas (1899) described the differences in detail, and came to the conclusion that the *R. pellio* of Schneider and that of Bütschli were separate species. Johnson (1913) found that by taking every precaution from allowing soil nematodes to contaminate an infected dead earthworm, only adults of the *R. pellio* of Bütschli occurred, while if strict precautions were not taken some of Schneider's forms occurred as well. Johnson thus considers the *R. pellio* of Bütschli to be the parasite of earthworms, while the *R. pellio* of Schneider is a free-living soil species.

III. Measurements.

Measurements (in mm.), see Table I, were made of adults, larvae and eggs. The nematodes were killed and extended by heating in water under a cover glass, and measured by means of a micrometer eyepiece.

Table I.

Adults, larvae and eggs	No. measured	Average length	Average diameter	Length largest individual	Length smallest individual
Adult ♂♂	11	1·24	0·07	1·81	0·99
Adult ♀♀	20	1·5	0·1	1·91	1·06
Nephridial larvae	22	0·59	—	0·69	0·42
Larvae recently emerged from cysts	28	0·66	0·04	0·7	0·52
Larvae recently emerged from eggs, or laid viviparously	14	0·33	—	0·36	0·28
Eggs	—	0·55	0·39	—	—

The measurements of the larvae and eggs closely approach those of Johnson (1913), but those of the average adult ♂♂ and ♀♀ from an earthworm are considerably smaller, Johnson having measured the largest individuals obtainable. Measurements of the F_1 generation, which were grown in various culture media, varied considerably according to the environment. In some cases newly hatched larvae were but 0·24 mm. long, while others, probably in their final moult, were only 0·7 mm. long.

IV. Culture media.

Considerable difficulty was experienced in culturing this nematode, and many of the more or less successful methods employed by Johnson (1913) and Potts (1910) did not work in this case. A mature F_2 generation was never obtained. The following culture media were tried which proved more or less successful at various times:

(1) A 0·2 per cent. solution of peptone.
(2) Johnson's earthworm broth (see Johnson, 1913).
(3) A soil extract.
(4) Thornton's bacterial count medium (used with and without agar).
(5) Nutrient agar.
(6) Nutrient gelatine.
(7) Pieces of non-infected body-wall of an earthworm submerged in tap water.
(8) Tap water to which had been added a little putrid fluid from a decaying earthworm, or bacteria from one of the above media.

No attempt was made at aseptic cultivation, the various sterile media being contaminated by bacteria through exposure to the air, or by the nematode itself. The nematodes were either isolated in hanging drop cultures, or placed individually or together in watch glasses with the various media. In many cultures the excessive growth of bacteria and a lack of oxygen were unfavourable for the development of the nematodes. This difficulty was partly overcome by using either (*a*) Thornton's medium (which prevents excessive bacterial growth), (*b*) tap water to which had been added a limited amount of fluid containing bacteria, or (*c*) media after the activity of putrefactive bacteria therein had subsided. Under natural conditions the nematodes were always found on the surface of the decaying earthworm, and if pushed into the decomposing mass on the point of a needle, they soon worked their way to the surface again so as to come in contact with the air.

V. Number of offspring produced per female, and the length of life in various media.

In order to determine the number of eggs laid, adult females were removed from a decaying earthworm as soon as possible when mature, usually after 4 days, and cultured in various media. The media were changed regularly, and

the total number of eggs, or larvae, counted (see Table II). Some of the females had doubtless laid a certain number of eggs or young previously.

Vivipary, in the case of the eight females cultured in the first three media (see Table II), was governed by conditions previously existing in the dead earthworm, for it occurred even in soil extract, a distinctly unfavourable medium. In only one other female did vivipary occur and that in a previous experiment in 0·2 per cent. peptone. In this female vivipary was entirely governed by cultural conditions, for it did not occur until 5 days after she was put in this medium, eggs being laid up to that time. Johnson (1913) found that females cultured in peptone were always viviparous, while in earthworm broth they were oviparous at first and later viviparous. He ascribes the phenomenon entirely to the nutritive quality of the food supply inducing the eggs to develop faster than they can be laid. A high temperature together with suitable conditions would be probably also favourable to vivipary.

Many other cultural experiments were made with adult (*F* generation) females taken from dead earthworms after 4–8 days, but the total number of

Table II.

Medium	Number of females	Average number of eggs or larvae	Maximum number of eggs or larvae	Average length of life	Oviparous or Viviparous
Soil extract	3	37	64	2½ days	Viviparous
Earthworm broth	3	47	53	6 days	Viviparous
0·2 % peptone	2	59	61	2½ days	Viviparous
Tap water + bacteria from an agar culture	3	118	157	18 days (max. 28)	Oviparous
Tap water + fluid from a decaying earthworm	2	174	257	6 days	Oviparous
Plain tap water	2	71	72	5 days	Oviparous

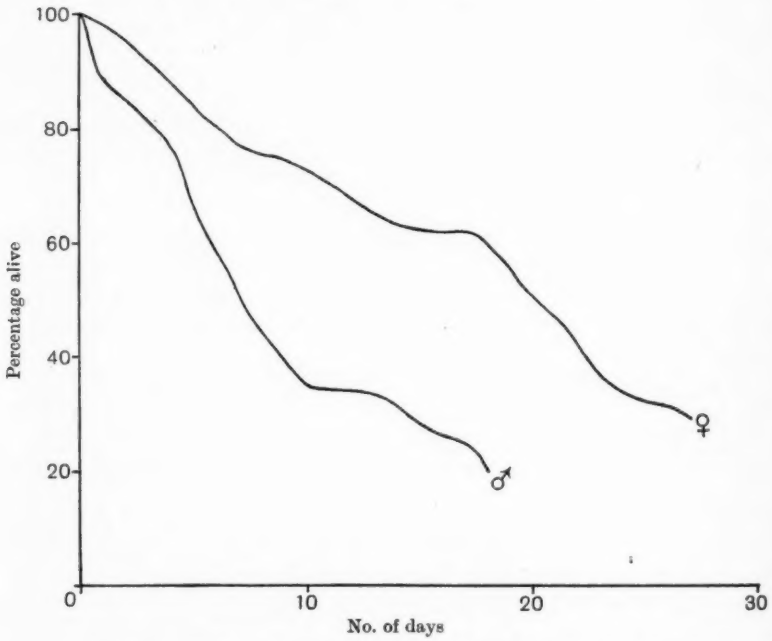
eggs laid per female was not accurately counted, and in many cases the females had probably laid many eggs before being submitted to experiment. Four females, however, cultivated in Thornton's medium, nutrient agar, nutrient gelatine and tap water with bacteria added from an agar culture, laid 140, 125, 159 and 160 eggs respectively, but these were only a few among many females cultivated.

Of many F_1 females, only one laid more than 25 eggs (197 eggs) in a hanging drop culture of Thornton's medium—she was alive at the end of 43 days. Judging from the number of eggs laid by this female compared with the number laid by other females of the *F* generation, the total number laid per female under natural conditions is probably 150–300.

The nature of the culture medium appeared to play an insignificant part in egg production. Placed in tap water, the females usually died rapidly from starvation, but ripe eggs were laid, and if the female died before all had been extruded, those remaining hatched *in situ*, and the larvae fed on their mother's decaying tissues (see Johnson, 1913). At times the larvae did not emerge from the old skin until after 7 days, and on emergence often averaged 0·4–0·45 mm.

in length. In most cases the females died after laying all their eggs. In the decaying earthworm, the females probably live but 7–10 days from the time they attain maturity, whilst the males probably survive for a very much shorter period. It was only in Thornton's medium (and in a few cases in tap water with nutrient liquid) that adults lived for longer periods.

With a view to determining the relative length of life of adult males and females under identical artificial conditions, 60 (*F*) ♂♂ and 70 (*F*) ♀♀ were cultivated separately in Thornton's medium (liquid), the medium being changed from time to time. Graph I shows the percentage of living ♂♂ and ♀♀ as long



Graph I. Showing the percentage of ♂♂ and ♀♀ alive in Thornton's medium.

as accurate results were obtained, and also that under cultural conditions ♂♂ live about a third as long as ♀♀. One ♂ however lived for 26 days, and one ♀ lived over 44 days.

Some F_1 ♂♂ and ♀♀ lived for long periods in changes of watery media or dilute earthworm broth: several ♀♀, besides the one ♀ which laid 197 eggs (see p. 299), lived over 30 days and one ♀ over 40 days. The ♂♂ usually lived 7–10 days, but one survived 35 days. All these F_1 ♂♂ and ♀♀ were bred from *F* ♀♀ in the same type of media but of stronger concentration, and usually attained maturity 8–10 days after hatching. Larvae, dissected but from a nephridium or recently hatched from cysts, were much more susceptible to

cultural conditions than those bred in cultures. All larvae, however, were more delicate than the adults.

The growth rate of F_1 larvae was slightly greater in earthworm broth than in 0·2 per cent. peptone, and on the whole it was found that the more natural media (pieces of dead earthworm in water or earthworm broth) were more satisfactory in quickening growth and maturity than the artificial media (peptone or nutrient agar), while Thornton's medium and dilute suspensions of bacteria were best for prolonging life. It seems that the longevity of adult ♂♂ and ♀♀ depends largely on the richness of the food supply, a decaying earthworm, or a medium rich in bacteria, accelerating the life processes and thereby bringing on old age and death quicker than a dilute medium or Thornton's medium, which is known to check bacterial activity. The failure of larvae to mature within the living earthworm is probably also due to partial starvation.

VI. Food of *Rhabditis pellio*.

In agreement with the results of Johnson (1913), it was found that *R. pellio* could grow and mature only in the presence of an optimum number of bacteria or their products. In nature the food of *R. pellio* larvae, which inhabit the earthworm's nephridia, probably consists of bacteria which are carried down in the excretory fluid. The bacterial flora of this fluid is comparatively scarce in the living earthworm, with the result that little or no growth of the larvae takes place and maturity is not attained, although there is sufficient food to keep the larvae alive. In the soil the larvae are under comparable conditions, few bacteria being present except in places where putrefactive processes occur and a suitable bacterial flora is available. The larvae are probably drawn by chaemotactic means to a suitable food supply in the soil, or to an earthworm by means of the secretion from the nephridia or coelomic pores. Starvation, on the other hand, may induce encystment, as Potts (1910) and Maupas (1899) found in the free-living species of *Rhabditis*. When the earthworm dies and is invaded by bacteria, the larvae escape from the nephridia or hatch from their cysts in the coelom. These larvae may feed, either on bacteria causing putrefaction in the earthworm, or on the earthworm's tissues which have been liquefied by the enzymes of bacteria. Johnson (1913) found that the F_1 larvae, after a time, tended to migrate through the soil away from a decaying earthworm, although adult males and females (*F* generation) might continue feeding on the earthworm's remains. I found, however, that the F_1 generation frequently became sexually mature upon the decaying earthworm, usually after 5–6 days. The number of generations developing on an earthworm probably depends on its size and the rate at which changes take place in the host owing to bacterial activity. It is possible that bacteria of different species may succeed each other in growing in a dead earthworm so that, after a certain time, by changing it chemically, it becomes repellent and eventually toxic to the larval nematodes. Johnson's observation of the migration away from a dead earthworm of these

larvae may be accounted for by their greater susceptibility to these conditions which may not affect the adults. Potts (1910), although finding the free-living species of *Rhabditis* most abundantly in places which were swarming with bacteria, considers that owing to the rareness of glandular cells in the intestine, the nematodes probably cannot digest the bacteria themselves, but feed by absorbing the soluble substances in the culture, which have been prepared by bacterial action. McCoy (1929), working with *Ancylostoma caninum* on the other hand, found that bacteria were the chief food of this nematode. His experiments show that the relative food values of various species of bacteria differ, when tested out as food for this nematode.

VII. Bisexuality and hermaphroditism in *Rhabditis pellio*.

Maupas (1899), working on free-living species of *Rhabditis*, describes three distinct sexual forms among species of this genus:

(1) *True bisexual species* with ♂♂ and ♀♀ in equal numbers.

(2) *Hermaphrodite species* of two kinds:

A. Hermaphrodites, in which one side of the gonad produces sperm and eggs, while the other side produces only unfertile eggs. True (functional) males (and perhaps females) occasionally occur, which were in some species found to refertilise the hermaphrodite females, which had exhausted their stock of sperm.

B. Hermaphrodites, in which the gonads produce first sperm, and later eggs, which are fertilised by these sperm. Males are rare and have lost their "sexual instinct."

(3) *Parthenogenetic species* in which males are unknown.

Maupas (1900) and Potts (1910) consider that the hermaphrodite species are derived from the bisexual species, the series terminating in the parthenogenetic species. Potts also found species showing intermediate stages, in which functional males and females occurred with the hermaphrodite females in varying proportions, which at times fluctuated regularly or irregularly according to some unknown factor.

All workers previous to Johnson (1913) considered *R. pellio* to be a bisexual species. Johnson, however, considers it to be an hermaphrodite species for the following reasons:

(1) The comparative rareness of males, one male to five females in the parental generation, after which, in subsequent generations bred in cultures, the male proportion usually dropped to nothing. Comparatively small numbers were however bred or determined for sex.

(2) Copulation, or refertilisation, was never observed.

(3) Spermatozoa were definitely observed in the gonad of one female.

In two of his experiments, however, the females behaved as if bisexual, but he does not attach much importance to this on account of an unsatisfactory

culture medium in the one case and the non-observance of copulation in the other.

Although a mature F_2 generation was never procured during this work, all the evidence obtained, regarding the question of sex of the F and F_1 generations, points to bisexuality:

(1) A few undoubted cases of copulation (or perhaps refertilisation of an hermaphrodite female) were observed among F generation males and females obtained from dead earthworms.

(2) In no case was a filial generation produced except from F generation females, which could have potentially come into contact with males before being experimented upon, or from F_1 females which were isolated with males in cultures.

(3) The sex ratio.

In the F generation the average ratio was 1 ♂ : 2·2 ♀♀, 4901 individuals being determined for sex (1532 ♂♂ and 3369 ♀♀), representing the totals obtained from 15 earthworms of various species, care being taken to eliminate any contamination from an F_1 generation. The highest ratio obtained was 1 : 5 and the lowest 1 : 1·3.

In the F_1 generation, the average ratio was 1 ♂ : 2·54 ♀♀, 959 individuals being determined for sex, being the offspring from 13 F females which were cultivated in various media. The highest ratio obtained was 1 : 5 and the lowest 1 : 1·27. There was one exceptional case in which the ratio was 1 ♂ : 10·6 ♀♀, and three cases in which the male proportion was equal, or slightly greater, than the female. These instances are not included in the average ratio.

No information was obtained regarding the effect of the various media on the sex ratio.

Although throughout this work *R. pellio* behaved as bisexual, Johnson, on the other hand, has obtained undoubted cases of hermaphroditism. *Rhabditis pellio* may thus be considered to be one of those species of *Rhabditis* in which hermaphroditism is in a very early stage, and in which functional males, females, and hermaphrodite females, exist side by side in fluctuating proportions.

VIII. Degree of infection of some British species of earthworms by *Rhabditis pellio*.

In order to determine the relative degree of infection of various species of earthworms and of the different regions of their bodies, specimens of *Lumbricus terrestris*, *Lumbricus rubellus*, *Octolasium cyaneum* and *Eisenia foetida* were placed, after being carefully washed, in changes of nematode-free soil for a fortnight before being killed, in order to eliminate any soil nematodes in their alimentary canals. After being killed by drowning, the earthworms were cut up into the following regions, which were allowed to decompose separately on nematode-free sand:

Region 1. Head (segment I to the beginning of the clytellum).

Region 2. The clytellum.

Region 3. Middle region (from the end of the clytellum to within about ten segments from the tail).

Region 4. Tail region (about the last ten segments).

The nematodes were removed from the various regions and counted after 4–5 days (see Table III), when the *F* generation was mostly mature, all larval stages (late developing *F* and F_1 generations) being rejected.

In Table III the degree of infection is given as the number of nematodes per segment of each region.

It was thought that the tail would prove to be by far the most heavily infected region of the body, on account of the additional presence of the encysted larvae among the "brown bodies," besides those inhabiting the nephridia. However, this was not so, for although the distribution of nematodes appeared haphazard in most cases, the tail was usually one of the least infected regions, showing that the proportion of encysted forms is comparatively small. There is an indication that the head region and clytellum are on the whole the most heavily infected.

Table III.

Species	No. examined	Average length and number of segments	Degree of infection				Average total No. of nematodes
			Head	Clytellum	Mid-region	Tail	
Lumbricus terrestris	2	85 segments 6–8 cm.	13·3	23·5	2·7	3	696 (73 & 1323)
Lumbricus rubellus	14	100 segments 8 cm.	1·25	0·93	0·93	0·89	95 (0–532)
Octolasium cyaneum	4	11 cm.	1·53	10·2	5·7		521 (125–753)
Eisenia foetida	26	Only one nematode found in the tail region of one specimen					

The degree of infection is possibly governed chiefly by contact with infected soil, but Johnson (1913) also considers that there is a possibility of young earthworms becoming infected from their mother when in the cocoon.

The rareness of infection of *Eisenia foetida* may possibly be due to the secretion from the skin glands stopping the entrance of the nematodes into the coelomic or nephridiopores, or to some unknown factor which causes the nematodes to migrate from these earthworms (see Johnson, 1913) while in the nematode-free soil. An excess of mucus secretion might at times also prevent the larvae from entering an earthworm.

Johnson found the nephridial larvae of *R. pellio* in all the species of earthworms mentioned in Table III, and also in *Dendrobaena subrubicunda*, the coelomic cysts in *Lumbricus terrestris*, *L. rubellus*, *Allolobophora longa* and *A. turgida*.

The only other species of nematode, besides *R. pellio*, which was found in the various species of earthworms examined, was a species of *Porrocaecum*, an Ascarid parasite of small mammals and birds. This nematode only occurred in a larval stage in *Lumbricus terrestris*, *L. rubellus* and *Eisenia foetida*, and

was probably being carried passively by these earthworms. This is probably the Ascarid mentioned by Shipley (1902) in his list of nematodes found in earthworms.

IX. Life history.

Having failed to find the larvae of *R. pellio* free in the soil (once found by Maupas, 1899), and failed to rear to maturity those F_1 larvae which had migrated away from a dead earthworm, Johnson (1913) tested the possibility of the existence of an intermediate host. He found, however, that in most cases the larvae were killed by the digestive juices of moles and birds, and those which survived were in the same stage of development as in the earthworm's coelom or nephridia. The larval *R. pellio* is most probably only free in the soil for very short periods, and over a very limited area, before being attracted to another host, or collecting around some other rich source of food, if not it probably encysts (see Potts, 1910, and Maupas, 1900).

The probable life history may be summarised as follows (see also Johnson, 1913):

(1) Eggs are laid in a decaying earthworm, or perhaps some other decaying flesh. These eggs hatch and the larvae may become mature and in turn lay eggs, the number of generations that the environment can support probably depending on its bulk, and the rate of bacterial activity.

(2) When the above environment is no longer favourable, the half-grown larvae migrate through the soil.

(3) Here they may:

A. Be attracted to some other source of food and become sexually mature.

B. Be submitted to conditions of drought, starvation (Potts, 1910), or perhaps a low temperature, all of which would probably induce the encystment of those larvae which survived.

C. Be attracted by the secretions from the nephridia, or coelom, to enter another earthworm, or some other suitable animal (Maupas, 1899, found the larvae of *R. pellio* in slugs in Algeria), by the various pores in the skin. If they work their way into the coelom they may be forced to encyst if attacked by amoebocytes as described by Keng (1895), or starved. In the nephridia, and perhaps in the genital organs, they live free. Food is scarce and little growth takes place.

(4) When within the earthworm they may either:

A. Be forced out of the coelom, or nephridia, by an excessive secretion of fluid, and passed back into the soil where they may possibly re-enter the same earthworm or another.

B. Or perhaps they may crawl out through some other cause.

C. Or be able to infect young earthworms by means of the cocoon.

(5) A bird, or some other animal, may eat the earthworm, when the larvae are freed, and if not killed by the digestive juices, are passed back into the soil. The cysts can probably survive the passage.

(6) The earthworm may die and decompose in the soil, when the larvae become sexually mature.

X. Summary.

1. Accurate measurements were made of adults, larvae and eggs of *Rhabditis pellio*, representing an average selection for comparison with Johnson's figures.

2. The most satisfactory method of culturing the nematodes was in hanging drops, in a medium free from an excess of putrefying bacteria.

3. A medium poor in food was found to prolong life (up to 40 days), while a rich medium quickened up the life processes thereby causing death sooner. It is probably partial starvation which prevents the larvae from becoming mature while in the living earthworm.

4. In a medium favourable for prolonging life, the males were found to live about one-third as long as the females (*F* generation). It is probable that the females only live for about 4–8 days in a decaying earthworm after attaining maturity.

5. The maximum number of eggs laid per female was 257 (*F* generation) and 197 (F_1 generation). It is probable that between 150 and 300 eggs are laid per female in the natural state. No information was obtained regarding the effect of the various media on egg production.

6. In most cases the females died when spent of eggs.

7. Vivipary is considered to be due to a favourable environment probably aided by a high temperature.

8. Larvae were found to be more susceptible to cultural conditions than the adults, especially those (*F* generation) which were not bred from females in culture media. The growth-rate of F_1 generation larvae was found to be slightly faster in a natural medium, such as earthworm broth, than in peptone.

9. The chief food is considered to be bacteria.

10. During this work *R. pellio* behaved entirely as a bisexual species. A few cases of copulation were observed, showing that some of the males had not lost their "sexual instinct."

11. *Rhabditis pellio* is considered to be a species in which hermaphroditism is just commencing and in which true males and females exist together with hermaphrodite females whose numbers fluctuate, the degree of hermaphroditism possibly bearing some relation to the sex ratio.

12. The sex ratio was about 1 ♂ : 2 ♀♀, in both the *F* and F_1 generations, but varied considerably. There were a few cases in which the male proportion was equal, or higher, than the female.

13. Four different species of British earthworms were examined, each being infected by *Rhabditis pellio* to a varying extent. *Eisenia foetida* was, however, rarely infected. Size of the earthworm, in proportion, played no part regarding the degree of infection. There is an indication that the head (segment I to the beginning of the clytellum) is the most heavily infected region.

14. A few specimens (larval stage) of an Ascarid, a species of *Porrocaecum*, a parasite of small mammals and birds, were found in three of the species of earthworms examined.

15. A probable course of the life history is given, based on this work and that of Johnson (1913).

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A NEW SPECIES OF THE NEMATODE GENUS *UNCINARIA* FROM A SEA-LION, WITH SOME OBSERVATIONS ON RELATED SPECIES.

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(With 9 Figures in the Text.)

Through the kindness of Dr S. W. Kemp, F.R.S., and of the *Discovery* Committee, the writer has recently had the opportunity of examining examples of a species of *Uncinaria* from the intestine of a sea-lion. These were collected by Mr J. E. Hamilton, of the *Discovery* staff, at Cape Dolphin in the Falkland Islands, in April 1931. The host was a sea-lion pup, about three months old, which was found dead on the beach, and it was suspected that the worms were concerned in the causation of its death.

The host, as the writer is informed by Mr Hamilton, is *Otaria byronia* (synonym, *O. jubata*). As a species of *Uncinaria* had already been recorded as a parasite of the fur-seal of the Pribilof Islands in the Bering Sea (*Callorhinus alascanus* [synonyms, *Callorhinus ursinus*, *Callotaria alascana*]), and had been considered the most important cause of mortality among young seal-pups in that region (see Lucas, 1899), it was at first thought probable that the Falklands species would prove to be the same. A very brief description of the Pribilof *Uncinaria*, based on inadequate material, was given by Stiles and Hassall (1899), and the species was subsequently named *Uncinaria lucasi* by these authors, in Stiles (1901). In spite of the large number of seals killed annually, no fuller description of this parasite than that given by Stiles and Hassall appears to have been published. The writer is indebted to Dr Maurice C. Hall, of the United States Bureau of Animal Industry, for information on this point. Dr Hall kindly lent all that remains of the original material for comparison. This consists of a slide on which are mounted (in balsam) several fragments of a ♂. Unfortunately none of the important characters could be made out in these fragments, and Dr Hall states that "the other specimens (two females) have long since dried up and are not available."

Inasmuch as the measurements of the Falklands specimens do not agree at all well with those given by Stiles and Hassall, the only possible course seems to be to describe this material as representing a new species. Together with the remains of the type ♂ of *U. lucasi*, Dr Hall was kind enough also to send for examination one ♂ and three ♀♀ of an *Uncinaria* obtained from a sea-lion at Palo Alto, San Francisco Bay, California, in 1901. These specimens agree very

closely with those from the Falklands, and it seems probable that they belong to the same species, though there are certain slight differences in their measurements, particularly in the length of the spicules in the ♂. The precise determination of the host is not given on the label. According to Bonnot (1929)[1] much the commoner of the two species of sea-lions occurring in the region of San Francisco Bay is *Eumetopias jubata* (synonym, *E. stelleri*). The other species is the Californian sea-lion, *Zalophus californianus* (synonym, *Z. gillespii*), the northern limit of whose range is reached at about this latitude. It seems highly probable, therefore, that the host of the Californian specimens was *Eumetopias jubata*.

The following description is based on the material from *Otaria byronia*, Falkland Islands.

Uncinaria hamiltoni, sp.n.

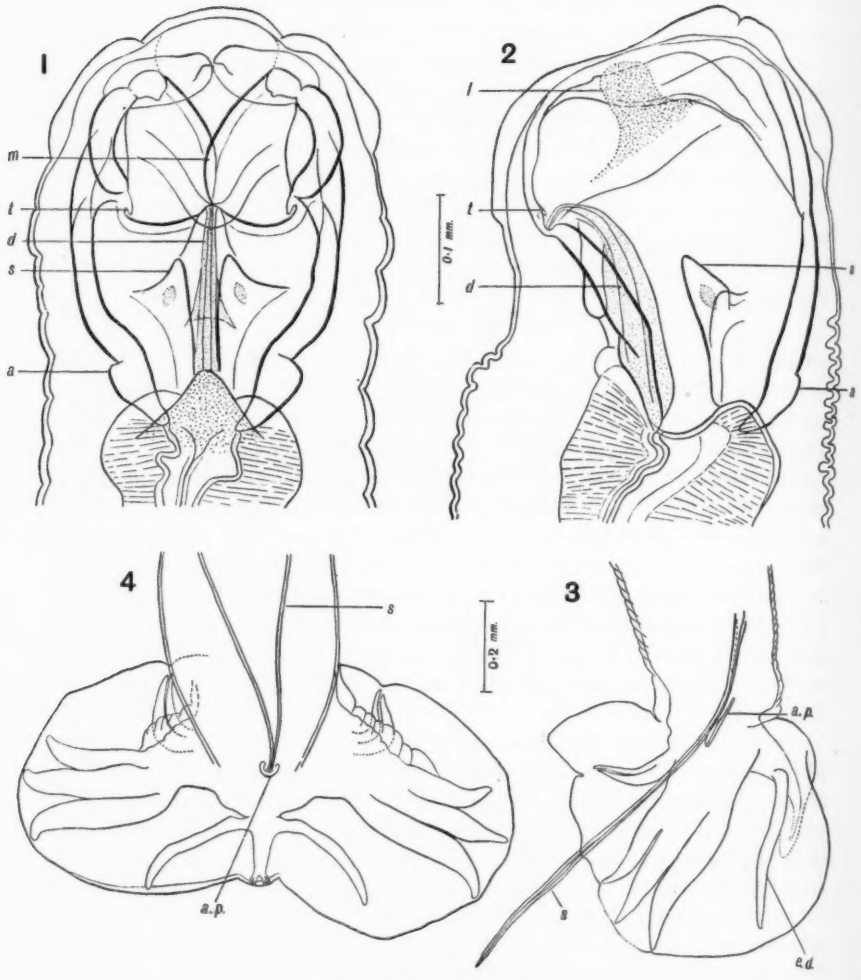
The ♂ measures 8·5–12 mm. in length and 0·4–0·46 mm. in maximum thickness, the ♀ 12·5–17·5 mm. and 0·52–0·6 mm. respectively. The length of the buccal capsule is about 0·28–0·3 mm. in the ♂ and 0·32–0·38 mm. in the ♀. The form of the capsule (Figs. 1 and 2) is very similar to that in *U. stenocephala*. There is, however, a more or less pronounced annular thickening of its wall (Figs. 1 and 2, *a.*) near the base. This is not equally conspicuous in all specimens, but some trace of it can generally be seen. The subventral teeth (Figs. 1 and 2, *s.*) are very well developed and prominent. At the subdorsal angles of the anterior border of the capsule there is a pair of structures (Figs. 1 and 2, *t.*) which have the appearance of small, backwardly-curved teeth. The oral aperture is generally dorso-ventrally elongate, and the edges of the cuticular membrane which bounds it tend to overlap at the dorsal end (Fig. 1, *m.*).

The distance from the anterior extremity of the head to the posterior end of the oesophagus is 1·5–1·55 mm. in the ♂ and 1·6–1·9 mm. in the ♀. The oesophagus is of the typical claviform shape. The cervical papillae are rather variable in position (0·47–1·1 mm. from the anterior extremity). The position of the excretory pore is also variable (0·6–1·1 mm. from the anterior end). The nerve-ring is slightly in front of the excretory pore.

There is nothing worthy of special notice in the structure of the bursa of the ♂ (Figs. 3 and 4). The main branches of the dorsal ray are tridigitate (Fig. 5) as in typical species of the genus. The outermost terminal branch is the longest. The spicules are slender, transversely striated and finely pointed, and, in all the specimens examined, almost exactly 1 mm. long. The accessory piece (Figs. 3 and 4, *a.p.*) is widened posteriorly, forming a wide groove for the spicules. It measures 0·125–0·137 mm. in length.

The tail of the ♀ (Fig. 6) is 0·16–0·25 mm. long. It has a terminal spike whose shape and length are rather variable. There is a pair of caudal papillae at about 0·08 mm. from the tip. The vulva is situated at 5·1–7 mm. from the

[1] The writer is indebted to Mr J. E. Hamilton for drawing his attention to this paper, and for valuable help in the matter of the nomenclature of the sea-lions.



Figs. 1–4. *Uncinaria hamiltoni.*

Fig. 1. ♀. Anterior end; dorsal view. *a.* annular thickening of wall of buccal capsule; *d.* duct of dorsal oesophageal gland; *m.* membranous border of mouth; *s.* subventral tooth; *t.* subdorsal "tooth."

Fig. 2. ♀. Anterior end; lateral view. *a.* annular thickening of wall of buccal capsule; *d.* duct of dorsal oesophageal gland; *l.* lateral papilla; *s.* subventral tooth; *t.* subdorsal "tooth."

Fig. 3. ♂. Posterior end; lateral view. *a.p.* accessory piece; *e.d.* externo-dorsal ray; *s.* spicules.

Fig. 4. ♂. Posterior end; dorsal view, with bursa spread out. *a.p.* accessory piece; *s.* right spicule.

posterior end. Its lips are usually very prominent, but in some cases the whole of the vulva is retracted so as to be actually in a depression of the body wall. The arrangement of the ♀ genital tubes appears to be as usual in the genus. The eggs are oval and relatively thick-shelled. They measure 0·135–0·138 × 0·085–0·093 mm. The development of their contents (in material preserved in formalin) seems sometimes to have proceeded *in utero* as far as the formation of a coiled embryo.

The specimens from California already referred to are slightly larger, on the average, than those just described. The single ♂ available is about 9·4 mm. long, while the ♀♀ vary in length from about 16·7 to 18·8 mm. The oesophagus, on the other hand, is shorter (distance from anterior extremity to posterior

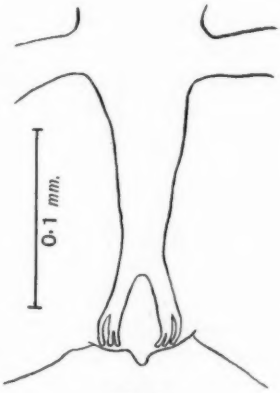


Fig. 5.

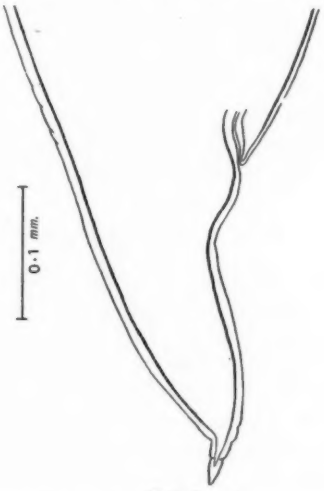


Fig. 6.

Figs. 5 and 6. *Uncinaria hamiltoni*.

Fig. 5. ♂. Dorsal ray of bursa; dorsal view. Fig. 6. ♀. Posterior end; lateral view.

end of oesophagus, ♂, 1·3 mm.; ♀♀, about 1·4–1·6 mm.), and the excretory pore is more anteriorly placed (at 0·45–0·55 mm. from the anterior end). The buccal capsule is, on the whole, a little smaller. Its length, in the ♂, is 0·22 mm., and in the ♀ 0·3–0·32 mm. The only striking difference, however, is in the length of the spicules of the ♂, which here measure only 0·67 mm. As only one ♂ was available for examination, it is doubtful how much importance should be attached to this character. Although the length of the spicules appears to be very constant in the Falklands material, there may be some variation in different localities, and it does not seem desirable to regard the Californian species as distinct until it has been more fully investigated.

In *Uncinaria lucasi*, according to Stiles and Hassall (1899), the ♂ is only 6·5 mm. long, the ♀ 12 mm., and the spicules 0·5 mm.

Other species of *Uncinaria*.

While studying the *Uncinaria* of sea-lions, the writer has taken the opportunity of examining the status of several other species of the genus. This has been rendered possible through the kindness of several correspondents, to whom grateful acknowledgment is here made.

Dr Maurice C. Hall, in addition to sending the sea-lion material already mentioned, very kindly presented to the British Museum (Natural History) paratype material of *Uncinaria philippinensis* Chitwood, and also examples of *U. lotoris* Schwartz and American specimens of *U. stenocephala* from dog and black fox. Some specimens of an *Uncinaria* from the European badger, determined by Prof. A. Henry as *U. criniformis*, were also lent by Dr Hall.

Prof. W. Arndt, of the Zoological Museum, University of Berlin, very kindly lent for examination several of Rudolphi's original specimens of "*Strongylus criniformis*," and also another specimen of *Uncinaria* from a badger.

To Dr P. A. Maplestone thanks are due for presenting to the Museum specimens determined by him as *Uncinaria longespiculum*. For European material from dogs and foxes the writer tenders his best thanks to Prof. J. Ciurea, Prof. F. Fülleborn, Prof. J. E. W. Ihle, Prof. C. Sprehn and Dr Hans Vogel.

Uncinaria stenocephala (Railliet 1884).

The question of the specific identity of various forms of *Uncinaria* from dogs, foxes and other carnivores has been discussed by several authors, *e.g.* by Looss, Fülleborn, Cameron and Ransom. Looss (1911) erected a species, *U. polaris*, for a form from the silver fox (*Vulpes lagopus*) obtained by Hassall in the United States of America, and considered it distinct from the form found in dogs in Europe, for which he uses the name "*U. criniformis* (Rud.)." In his earlier paper (1905) he had stated that the original *Ascaris criniformis* of Goeze 1782, from the badger (*Meles meles* [*M. taxus*]), was an unidentifiable species, and that its type specimens were no longer available. The original specimens of Rudolphi's *Strongylus criniformis*, on the other hand, preserved in the Berlin Museum, were re-examined by Railliet, who considered the species identical with his *U. stenocephala*. From Rudolphi's description it appears, as Looss (1905) points out, doubtful whether Rudolphi's specimens were of the same species as those of Goeze. We therefore do not know what Goeze's species was, though we do know that a species similar to, or identical with, that of the dog occurs in the badger.

Ransom (1924), having examined specimens from European dogs, American silver foxes and "several lots" from *Meles*, arrived at conclusions opposite to those of Looss. He considered *Uncinaria polaris* Looss a synonym of *U. stenocephala* (Railliet), but regarded the badger species as distinct from both, and used for it the name "*Uncinaria criniformis* (Goeze, 1782)." He states that Looss (1911) "redescribed *Uncinaria criniformis* originally reported by Goeze in 1782 from the European badger." In view of what has been said above this

statement is somewhat misleading. It is by no means certain that what Looss described was Goeze's species.

The writer has recently examined a considerable amount of *Uncinaria* material from dogs, foxes and badgers. The specimens examined included the following:

Nine batches from dogs (four from Roumania, three probably from Germany, one from Holland, one from Alaska).

Two batches from wolves (*Canis lupus*)—one from Roumania and one, a single ♀, probably from Germany (originally in v. Siebold's collection).

Twelve batches from silver foxes (*Vulpes lagopus*)—three batches from a fox-farm in Great Britain, one from a fox probably imported from Canada, one from Maine, U.S.A., one from Roumania and six from German fox-farms.

Three batches from red foxes (*Vulpes vulpes*)—two from Germany and one from Roumania.

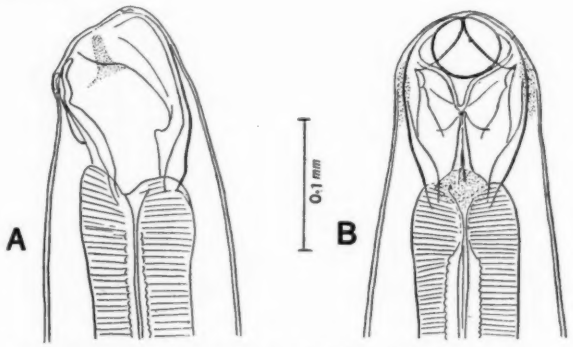


Fig. 7. *Uncinaria stenocephala* ♀. Anterior end of specimen from dog. A, lateral view; B, dorsal view.

Three batches from the European badger (*Meles meles*). One set of specimens came originally from the collection of Prof. A. Railliet, and were collected by Prof. A. Henry in France. A second set were part of the original specimens of *Strongylus criniformis* Rudolphi 1802, from the Berlin Museum (Catalogue No. 271). The third consisted of a single ♂ (Catalogue No. Q2494), also from the Berlin Museum.

The study of all this material has led the writer to the conclusion that all the specimens belong to one and the same species. Fülleborn (1924), Cameron (1924), Ransom and others were, in the writer's view, justified in regarding *U. polaris* as identical with *U. stenocephala*. This appears, indeed, now to be the generally accepted view. At one time the writer was inclined to think that differences (Figs. 7 and 8) in the shape of the oral aperture and of the buccal capsule, in the curvature of the neck, and in the shape of the boundary line between the thicker ventral and thinner dorsal portions of the buccal capsule might be of specific significance. All these characters, however, prove to be too

variable to be of any importance. The variations observed seem to be partly the result of actual individual differences between specimens, but much more usually the result of slight differences in the angle from which the specimen is seen under the microscope (cf. Fülleborn, 1924), and the amount of pressure exerted upon it by the cover-glass.

Ransom, in separating his material from badgers from the rest as a distinct species, relied partly upon measurements (which again are too variable to be of decisive value), partly upon differences (which were probably subjective and

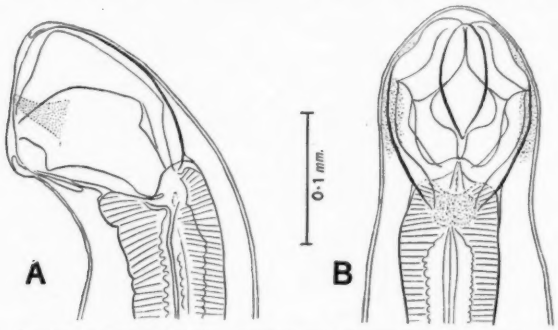


Fig. 8. *Uncinaria stenocephala* ♀. Anterior end of specimen from *Vulpes lagopus*. A, lateral view; B, dorsal view.

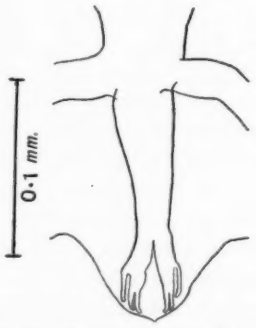


Fig. 9. *Uncinaria stenocephala* ♂. Dorsal ray of bursa of specimen from *Meles meles* (U.S. Bureau of Animal Industry No. 24,738); dorsal view. The edge of the dorsal lobe is somewhat curled under.

accidental) in the form of the buccal capsule and its parts, and partly on one observation which may have been erroneous. He states that the dorsal ray of the bursa of the ♂ is "bifurcated distally, each branch bidigitate," and gives a figure in which this is clearly shown. The label of the specimens from the U.S. Bureau of Animal Industry examined by the writer bears the number "24,738." One of the batches studied by Ransom appears to have been numbered "24,788," and it may be that these are actually part of his material. In any case, each of the two ♂♂ seen by the writer has a dorsal ray (Fig. 9)

the branches of which are distinctly tridigitate, as in all the specimens seen from dogs, wolves and foxes. The same is true of Rudolphi's specimens of "*Strongylus criniformis*," and of the other specimen from the Berlin Museum, referred to above. It is evident, therefore, that the bidigitate character of the dorsal ray is not constant in all specimens of *Uncinaria* from the European badger, and until a large series of specimens has been examined it must remain doubtful whether there exists in the badger a species of *Uncinaria* distinct from that of dogs and foxes. At all events it is clear that, as Railliet has already stated, a form does occur in badgers which is indistinguishable from the species now generally known as *U. stenocephala*.

In this connection it is interesting to note that Cameron (1924) has figured a dorsal ray of *U. stenocephala* in which one branch has three digitations, the other only two. He states that each branch of the ray redivides "into two digitations, the inner of which, as a rule, divides again. Sometimes, however, only one redivides." Cameron's material was obtained from a British fox (presumably *Vulpes vulpes*) which died in the London Zoological Gardens. Alessandrini (1905) describes, under the name of *U. criniformis* (Goeze), a form from *Meles* in Italy, in which the dorsal ray has no less than "eight to ten" terminal digitations, and gives a figure in which eight are shown. If this be, as appears probable, a genuine *Uncinaria*, the question of the multiplicity of species in badgers is still further complicated.

Uncinaria lotoris Schwartz 1925.

This species, recorded by Schwartz (1925) from a raccoon (*Procyon lotor*) in Maryland, U.S.A., seems to have quite distinctive characters. The writer has examined specimens kindly presented by Dr M. C. Hall to the British Museum (Natural History), and has nothing to add to the description given by Schwartz. The specimens in question were not part of the original material, but were collected from the type host in Oregon by S. N. Shaw, and identified by Dr E. W. Price.

It may be mentioned here that Dikmans[1] has doubtfully referred to *U. lotoris* a single ♀ of *Uncinaria* found in a skunk (*Mephitis mesomelas*).

Uncinaria longespiculum Maplestone 1931.

Synonym, *Uncinaria philippinensis* Chitwood 1932.

Maplestone (1931) described *U. longespiculum* from a civet-cat (*Viverricula malaccensis*) in the Calcutta Zoological Gardens. Chitwood (1932) described *U. philippinensis* from a Philippine civet (*Paradoxurus philippinensis*) in the National Zoological Park, Washington, U.S.A. The writer has had the opportunity of examining a pair of paratypes (♂ and ♀) of *U. philippinensis*, presented to the British Museum (Natural History) by Dr Maurice C. Hall, and also several specimens from the type host, identified as *U. longespiculum* by

[1] *J. Parasitol.* Urbana, **18**, 56 (1931).

Dr P. A. Maplestone, and presented by him to the Museum. As a result of this examination the writer is satisfied that the two species are identical. A remarkable peculiarity, not mentioned by either of the describers, is the presence, in the ♀, of a conspicuous, asymmetrical, subventral papilla at the level of the vulva on one side only. This papilla may be either on the left or on the right side, and was observed in both sets of specimens.

The name *Uncinaria longespiculum* has priority, and *U. philippinensis* must accordingly fall into synonymy.

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ON THE EXCRETORY SYSTEM AND LIFE HISTORY OF *LECITHODENDRIUM CHILOSTOMUM* (MEHL.) AND OTHER BAT TREMATODES, WITH A NOTE ON THE LIFE HISTORY OF *DICROCOELIUM DENDRITICUM* (RUDOLPHI).

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(With Plate XXI, containing Figs. 1–6, and Fig. I in the Text.)

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I. Introduction.

The European insectivorous bats possess a well-defined trematode fauna comprising the genera *Crepidostomum*, *Plagiorchis* and *Lecithodendrium*, all of which belong to families where Coleopterous, Ephemeropterous, Trichopterous and Dipterous insects serve as second intermediate hosts. As these insects constitute the greater part of the food of insectivorous Chiroptera it is remarkable that so little is known of the life history of bat trematodes. A few observations, which will be discussed later, have been made, but as far as I know only one of them can be substantiated. The genus *Lecithodendrium* is represented in Great Britain by two species, one of which, *L. chilostomum*, occurs as an intestinal parasite in nine of the ten species of bats indigenous to this country. Skriabine (1915) found sexually mature specimens of *L. chilostomum* containing eggs in the uterus, encysted in the body cavity of a single adult caddis fly, *Phryganea* sp., and concluded that this trematode attained sexual maturity in its intermediate host. Skriabine did not realise that this is essentially an example of progenesis in a metacercaria, neither did he ascertain

whether the eggs would produce miracidia. During a trematode survey of the River Nene (Northamptonshire) I obtained several larvae of *Phryganea grandis* and found in the body cavity a relatively large number of mobile early metacercariae which appeared to belong to the genus *Lecithodendrium*. Later, pupae and imagos were obtained in which more advanced stages were found, but no example of progenesis was discovered. The life and growth of the larvae is so unusual for larval trematodes that I am giving an account of it now, although I have not yet been able to establish the first intermediate host.

II. The life history of *Lecithodendrium Chilostomum*.

In the following account the larval stages are presented in their natural sequence in order to show their inter-relationships and the gradual assumption of adult characters.

(1) *Cercaria*.

During October and November, larvae of *Phryganea grandis* contained numerous unencysted early metacercariae of various sizes and among them several which possessed a stylet and part of the salivary glands. It was obvious that these had only recently penetrated the second intermediate host, and apart from the tail, which is lost during penetration, still retained distinctive features of the cercaria, so that it is possible to predict the structure of the latter with tolerable accuracy.

The body is unspined and oval; 0·165 × 0·13 mm. The oral sucker (diameter 0·06 mm.) is twice the size of the ventral sucker, which is situated just behind the middle of the body. A slender and pointed bullet-shaped stylet, 0·037 mm. long and possessing a slight shoulder about one-third of its length from the tip, lies dorsal to the oral sucker. In some cases the stylet point is bent at an obtuse angle to the shaft, but I am inclined to believe that this is an abnormal state preparatory to disintegration. On each side of the stylet is the opening of a single duct from a salivary gland cell situated antero-lateral to the ventral sucker. This pair of cells is the remnant of three or possibly four such cells, the others having discharged their contents during penetration of the intermediate host. A small pharynx and short oesophagus are present and the latter soon bifurcates into the short club-shaped intestinal caeca which lie transversely to the body axis and end wholly in front of the ventral sucker.

The excretory vesicle is V-shaped and conspicuous, the arms extending to the sides of the ventral sucker; the excretory pore is terminal. Slightly anterior to the middle of the body the arms of the vesicle narrow, become convoluted and then bifurcate into anterior and posterior lateral ducts, each duct receiving capillaries from six flame cells arranged in three definite groups of two. The second and third groups on the anterior lateral duct and the first and second groups on the posterior lateral duct lie close together. The total number of flame cells is 24; the formula may be expressed as

$$2\{(2+2+2)+(2+2+2)\}.$$

The genital system is undeveloped and there are no signs of cystogenous gland cells. The only other organs which we should expect to find in the free cercaria are additional salivary gland cells and the tail; the latter will in all probability be a slender one about as long as the body. We should also expect this type of cercaria to develop in short sausage-shaped sporocysts in the digestive gland of a fresh-water Gastropod; probably one of the following which are associated with the larvae of *Phryganea grandis* in the River Nene: *Limnaea stagnalis*, *L. pereger*, *Planorbis corneus*, *P. carinatus*, *Viviparus viviparus*, *Bithynia tentaculata* and *Physa fontinalis*.

(2) *Metacercaria.*

It is usual for all previously known stylet cercariae to encyst in some tissue or organ of the second intermediate host immediately after penetration, and after some time the stylet is shed and eventually disintegrates within the cyst. *Cercaria Lecithodendrii chilostomi*, however, does not encyst at this stage, but the stylet is shed, the salivary gland cells disappear completely and the larva lives freely within the body cavity of the host. In many cases it attaches itself to the fat body which is pale green in *Phryganea grandis* larvae. The metacercaria feeds actively on some part of the tissue or body fluid of its host; yellowish green oil globules may be seen in the gut of the worm and the contents stain a bright red with neutral red, indicating a marked acidity which is probably produced during the process of digestion. During October and November there is not much variation in the size of the larvae, but growth takes place slowly during the winter months and large metacercariae are found during the following March, April or early May. These large metacercariae show a distinct advance in development over the early forms. The body, still unspined, is now pear-shaped, 0·51 × 0·39 mm.—the oral sucker has a diameter of 0·13 mm.—ventral sucker 0·06 mm., and pharynx 0·02 × 0·04 mm. The excretory system is exactly the same as in the cercaria. On the other hand, the genital system is well developed though not yet functional. The male organs are the first to develop and the testes make their appearance even when the larva is only 0·307 mm. long. The testes are round, a little smaller than the ventral sucker, and lie on each side of the body at the same level just anterior to the ventral sucker. The vasa deferentia run towards the middle of the body and join immediately before entering the cirrus pouch which lies directly in front of and almost contiguous with the anterior rim of the ventral sucker. Inside the pouch the vas deferens dilates to form a much convoluted seminal vesicle which opens at the genital pore. The female system does not begin to develop until the larva is about 0·35 mm. long. The round ovary varies slightly in position, usually it is to one side and partly posterior to the ventral sucker; the oviduct, receptaculum seminis, Laurer's canal, ootype and uterus are distinct in the larger larvae. The vitellaria lie in two fairly compact masses on each side of the pharynx and the vitelline ducts run posteriorly, towards each other, dorsal to the ventral sucker. They meet and form a small yolk reservoir

before entering the oviduct immediately anterior to the ootype. This metacercaria has nearly all the morphological characters of the adult, but the genital system is not yet functional.

(3) *Cyst and excysted form.*

The life as a metacercaria is not complete when it has reached the stage just described, and a further phase is entered during the pupal and imaginal stage of *Phryganea grandis*. The metacercariae migrate from the abdominal body cavity of the caddis larva and enter the thoracic region of the same larva during pupation. Here in the muscles of the thorax encystment takes place. The cysts vary considerably in shape from roughly spherical to oval or pear-shaped; from a diameter of 0·23 mm. to 0·23 × 0·21 mm. or even slightly larger. The cyst wall is very thin, but a delicate adventitious outer, almost spherical, cyst is also secreted, probably by the insect host. The excysted young trematode is also larger than the largest free metacercaria and the male genital organs are now functional, motile spermatozoa being present in the seminal vesicle. The body is also armed with extremely fine minute spines. The cysts are also found, as we should expect, in the imago and the excysted worm in this case is identical with that taken from pupal cysts. Although 21 pupae and imagines were examined and some 300 cysts obtained, in no case were there any eggs in the uterus, so that Skriabine's (1915) example of progenesis is probably an isolated one.

III. The excretory system and convergent evolution.

The importance of this system in determining genetic relationships in the Trematoda has been recognised and generally emphasised since the work of Looss (1894), and it is of considerable value in morphological life-history studies. In the majority of trematodes there is an increase in the number of flame cells and subsidiary ducts from the cercariae stage through the metacercaria to the adult. Unfortunately, very few cases are known where this trend has been followed completely in all three stages, but the system in *Allocreadium isoporum*, where two stages are known, may be quoted. According to Looss (1894) *Cercaria isopori* is the larval stage of *Allocreadium isoporum* (Fam. Allocreadiidae); the excretory system of the cercaria is of the 2 (4 × 2) type and in the adult is 2 (6 × 4) so that the subsidiary ducts have increased by 50 per cent. and the flame cells in a group by 100 per cent. This type of increase has led to the supposition that it is possible to arrange cercariae in natural groups on this basis alone, but it is clear that, in attempting to solve the wider problems of relationships and evolution of both larval and adult forms it is impossible to rely entirely on one system, other organs or systems must be considered. The nature and extent of the excretory system of *Lecithodendrium chilostomum* is of considerable interest in that it throws some light on both these problems. A system of the 2 (6 × 2) type serves the excretory function of cercaria, metacercaria and adult, and is one of the exceptions to the general rule that there is

an increase of units and subsidiary ducts throughout development. Another exception is *Cercaria vitrina*, which appears to be the larval form of *Dicrocoelium dendriticum* (Fam. Dicrocoelidae). This cercaria, according to Vogel (1929), has an excretory formula of $2\{(2 + 2 + 2) + (2 + 2 + 2)\}$ or $2(6 \times 2)$, and Faust (1929) has shown that the adult possesses exactly the same. On the other hand, except for the presence of a stylet, there is no further similarity in the organisation of *Cercaria vitrina* (= *C. Dicrocoelii dendritici*) and *C. Lecithodendrii chilostomi*. The salivary gland cells are entirely different in shape, extent and number, and although the tail of the latter is unknown it must be a

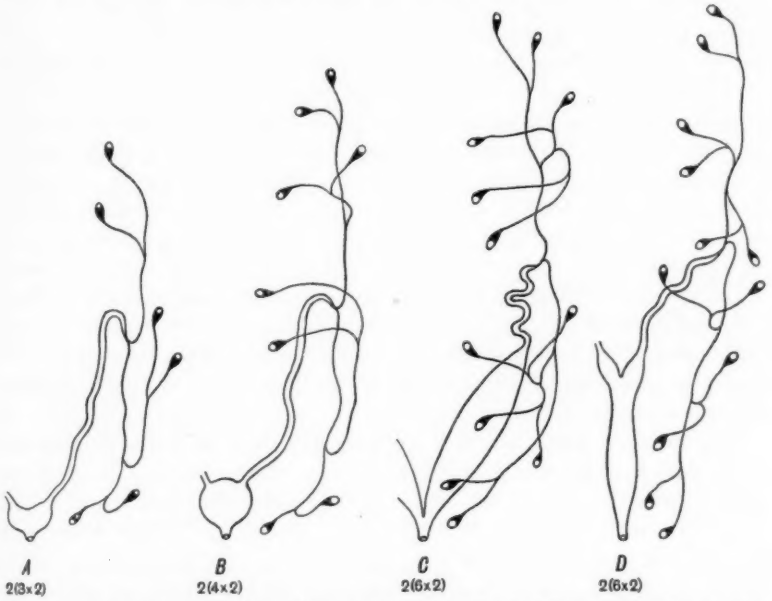

Fig. I. To show excretory vesicles and increase of groups of paired flame cells in the excretory system in *A*, Cercaria of "Parapleurolophocerca" group (after Sewell); *B*, Cercaria of "Isopori" group (after Sewell); *C*, *C. Lecithodendrii chilostomi* (original); *D*, *C. vitrina* (=*C. Dicrocoelii dendritici*, after Vogel). Only half of the system is shown and all figures are semi-diagrammatic.

swimming type, whereas in the former it is long, slender and not of this type. Even within the excretory system there is a difference in the shape of the excretory vesicle (Fig. I *C*, *D*).

With regard to the relationships of cercariae, Sewell (1922) has attempted to show that these may be deduced from the similarity or "increasing complexity" of the excretory system and other organs. In his "Pleurolophocerca," "Parapleurolophocerca" and "Isopori" divisions the flame cells have the same grouping and are arranged in pairs; the number of groups increases from three in the first to four in the last, and he states that since an adult trematode,

Microphallus opacus (Fam. Heterophyidae), possesses the latter grouping, relationship between the "Isopori" division and this form is extremely probable. Since *Cercaria Lecithodendrii chilostomi* (Fam. Lecithodendriidae) and *C. Dicrocoelii dentritici* possess six groups of paired flame cells, we have a continuous series beginning with the "Pleurolophocerca" group (see Fig. I) of systems 2 (3 × 2), 2 (3 × 2), 2 (4 × 2), 2 (6 × 2) which, according to Sewell, should express close relationship, and, by implication, the families to which these members belong, namely, the Allocreadiidae, Heterophyidae, Lecithodendriidae and Dicrocoelidae respectively.

A study of the shape and extent of the excretory vesicles and the reproductive systems will show, however, that these families are not by any means closely related. In addition, it is clear from Sewell's figures (1922, p. 325) that the "Pleurolophocerca" division, though agreeing to some extent in other larval features, possesses a different type of excretory vesicle and main collecting ducts from the "Parapleurolophocerca" and "Isopori" divisions, and since the connecting ducts of the flame cells are omitted by Sewell, the type of system could be either 2 (3 × 2) or 2 (2 × 3). I am convinced that, although the "Parapleurolophocerca" and "Isopori" divisions may be related, the "Pleurolophocerca" group is not related to either of them any more than is *C. Lecithodendrii chilostomi* or *C. Dicrocoelii dendritici*, and that the similarity of groups of flame cells and the number of groups does not necessarily imply relationship. It is clear that this condition must have arisen independently on several occasions during evolution within the Trematoda and is an example of convergent evolution.

The two main points which emerge from a study of the excretory system of *Lecithodendrium chilostomum* are (1) an increase in the number of units and subsidiary ducts is not a constant feature in the development from larval to adult trematodes and that when it does occur, is probably an expression of the physiological requirements of the organism, and (2) the similarity of the number of units in a group and the number of groups within the system does not necessarily denote relationship, but may be due to convergence.

IV. Biology of parasite and host.

The images of *Phryganea grandis* emerge during May and June, and egg-laying follows. The larvae attain a length of 2–3 cm. by October when they were first obtained. The number of metacercariae in each individual varies from 11 to 193, and in October and November they are of two types. About 10 per cent. of them are approximately 0·165 mm. long and retain the stylet and part of the salivary gland system of the cercaria; the rest are approximately 0·3 mm. long and have lost the characteristic features. It is obvious that the first type have only recently penetrated the Phryganid larvae and that there must be a swarming of cercariae from the molluscan host during October and November; but since 90 per cent. of the metacercariae are nearly twice as

large as the cercariae there must be an earlier and probably maximal swarming period. Very little is known of the swarming of cercariae under natural conditions, but in the laboratory heavy swarming may be induced usually by raising the temperature of the water containing the molluscan host. It is possible, however, to determine the period approximately in this case by assuming that the rate of growth of the metacercariae is constant over the winter months, when the temperature of a slowly flowing river (the habitat of *P. grandis* larvae) is fairly constant. From November to the following April, a period of approximately 18 weeks, the metacercariae increase in length from 0·16 to 0·51 mm. (see Table I). This corresponds to a weekly increase of about 0·017 mm. It follows that metacercariae 0·3 mm. long have taken approximately 18 weeks to reach this stage, so that the maximum swarming period of the cercariae would be about the end of June or the beginning of July. Since the rate of growth is usually correlated with temperature, and in the case of a parasite also with the metabolic activity of its host, this estimate is subject to a correction, since the temperature and metabolic activity will be greater during

Table I. *Showing growth of larval stages of* L. chilostomum.

	Mobile forms in body cavity of larva of *Phryganea grandis*			Encysted form in thorax of pupa and imago.
	Cercaria (Oct.–Nov.)	Metacercaria (Nov.–Apr.)		Young adult (Apr.–May)
Body length	0·16 mm.	0·307 mm.–0·51 mm.		0·60 mm.
Body width	0·113 mm.	0·205 mm.–0·39 mm.		0·31 mm.
Oral sucker (o.s.)	0·05 mm.	0·08 mm. –0·13 mm.		0·16 mm.
Ventral sucker (v.s.)	0·026 mm.	0·041 mm.–0·065 mm.		0·08 mm.
Ratio o.s.:v.s.	2:1	2:1	2:1	2:1
Testes	—	Present	Present	Present
Ovary	—	—	Present	Present
Vitellaria	—	—	—	Present
Excretory system	2 {(2+2+2)+(2+2+2)} in all stages			

the summer. The maximum swarming probably occurs during July. Another piece of indirect evidence in support of this may be adduced from the fact that larval trematodes are rarely found in very young stages of their respective hosts, and before this period the larvae of *P. grandis* are probably too young for successful infection. After the maximum swarming period there is a prolonged period of intermittent and decreasing emergence until sometime during November, when it falls off completely. No stylet-bearing metacercariae are found in the second intermediate host after November, but it would appear that there is a slight differential growth rate since a few undersized forms are invariably found in each infected host. The position of the metacercariae in the body cavity is remarkable; the majority of worms live in the three posterior segments of the host (see Table II). Here they remain until the formation of the pupa when they migrate into the thorax and encyst in the thoracic muscles, where they are also found in the imago. Since the emergence of the imago is confined to May and June, this is the period during which bats will become infected with *Lecithodendrium chilostomum*. I have found that another caddis-

fly, *Limnophilus rhombicus*, may also serve as a second intermediate host, so that the above infective period may be extended. On the other hand, the number of metacercariae in *L. rhombicus* is small (2–11) and as far as I have been able to ascertain from an examination of 37 imagos they do not encyst, neither do they always migrate to the thorax. It is also possible that these unencysted forms may be unable to withstand the digestive juices of the final host.

No other second intermediate hosts have been found and it would appear that *Phryganea grandis* is the normal one. The life cycle of this insect is approximately 11–12 months; the evidence adduced points to a first infection with *Cercaria Lecithodendrii chilostomi* at the age of 3 months, and subsequent infection up to 6–7 months. Thus the duration of life of the free metacercariae is from 5 to 8 months and that of the encysted form varies from the duration of pupal life up to 2 months, but the life at this stage depends mainly on the factors governing the emergence and flight of the insect and the activity of the final host.

Table II. *Showing number and position of metacercariae and cysts in* Phryganea grandis.

	Larva		Pupa		Imago	
	Last 3 segments	Rest of body	Abdomen	Thorax	Abdomen	Thorax
Oct.	178 Mmc.	12 Mmc.	—	—	—	—
Nov.	59 Mmc.	8 Mmc.	—	—	—	—
Dec.	83 Mmc.	7 Mmc.	—	—	—	—
Mar.	17 Mmc.	2 Mmc.	—	—	—	—
Apr.	35 Mmc.	3 Mmc.	3 Mmc.	63 C.	—	—
May: (1)	—	—	—	—	2 Mmc.	31 C.
(2)	—	—	—	—	—	48 C.

Mmc. = mobile metacercariae. C. = cysts.

V. Life history of other bat trematodes.

With the accumulation of morphological data of larval trematodes and increased knowledge of the biology of the intermediate and final hosts it is possible to indicate the life history of trematodes, especially in the case under consideration where the food of the final host is confined to winged insects.

Lecithodendrium lagena is the other member of this genus which occurs in most European bats. Braun (1879–93) states that *Cercaria armata*, which develops in sporocysts in the digestive gland of *Limnaea stagnalis* and encysts in "Ephemera" and "Perla" larvae, according to von Siebold, and the larva of *Chironomus plumosus*, according to von Linstow, is the larval stage of this form. On the other hand, Lühe (1909) states that it is doubtful whether "Ephemera" and "Perla" larvae are the second intermediate hosts of *Cercaria armata*, and in his description of *Lecithodendrium lagena* does not refer to this cercaria, but only states that the encysted form is found in *Chironomus*

plumosus. In 1926 I described *Cercaria pseudarmata*, which has the same molluscan host and encysts in the posterior segments only of the larvae of *Chironomus plumosus*, and suggested that since this form agreed closely with the sucker ratio of *Lecithodendrium lagena*, whilst *Cercaria armata* does not, *C. pseudarmata* was more likely to be the larval stage. That the sucker ratio is of value in morphological life-history studies is confirmed by the study of *Lecithodendrium chilostomum* (see Table I, p. 323), but in spite of this I do not now believe *Cercaria pseudarmata* to be the larval stage of *Lecithodendrium lagena*. When this cercaria was described, the excretory system of the Lecithodendriidae was unknown, and I assumed, since the two families are related, that it would be similar to the Plagiorchiidae. Now that I have been able to determine the excretory system of *L. chilostomum*, it is no longer possible to hold this view. In the Plagiorchiidae the flame cells occur in groups of three (*Opisthoglyphe*, 2 (6 × 3), *Haplometra*, 2 (6 × 3); Looss, 1894), whilst in the Lecithodendriidae there are only two cells in each group.

Also, the excretory vesicle is usually V-shaped in the Lecithodendriidae, and Y-shaped in the Plagiorchiidae. Although I was not able to determine the complete excretory system of *Cercaria pseudarmata* (see Brown, 1926, Pl. II, fig. 20), there is evidence to show that the groups consist of three cells, and the excretory vesicle is Y-shaped, so that this form is more closely related to the Plagiorchiidae. Now *Plagiorchis vespertilionis* is an insectivorous bat parasite and *Cercaria pseudarmata* may be the larva of this form, but further work is necessary to substantiate this. It can also be shown tentatively that if relationships between cercariae and molluscan hosts are examined, it will be found that the majority of stylet cercariae with relatively short tails and with V- or Y-shaped excretory vesicles develop in sporocysts in the digestive gland of the various species of *Limnaea*, so that it is highly probable that the molluscan hosts for *Lecithodendrium chilostomum*, *L. lagena* and *Plagiorchis vespertilionis* will be members of this genus.

Another trematode, *Crepidostomum moeticus*, is also a parasite of insectivorous bats, and since the life history of *C. farionis* has been determined (Brown, 1927), it is highly probable the life history of *C. moeticus* will be similar. The stylet cercaria will possess eye spots and will develop in rediae in a fresh-water lamellibranch, a species of the genus *Pisidium* or *Sphaerium* and the second intermediate host will probably be a mayfly.

Although this does not exhaust the trematode fauna of insectivorous bats, no information can be adduced with regard to other forms beyond the statement that the second intermediate host will be the larva of a winged insect. Recently, Macy (1931) has discovered and described a new species of *Dicrocoelium* from the gall bladder and bile ducts of the North American insectivorous bat *Tardarida cynocephala*, so that the following note is not out of place in this paper.

VI. Note on the life history of *Dicrocoelium dendriticum* (Rudolphi).

Several authors, Nöller (1929), Vogel (1929) and Cameron (1931), have attempted to establish the life history of this trematode, parasitic in the bile ducts of sheep. The larval stage appears to be *Cercaria vitrina* which develops in sporocysts in the small terrestrial molluscs *Zebrina detrita*, *Torquilla frumentum* and *Helicella candidula* on the Continent and *Helicella itala* in the Hebrides, Scotland. The eggs of *Dicrocoelium dendriticum* have been fed to these snails and *Cercaria vitrina* has been obtained, but in all cases so far recorded either the absence of control experiments, or the appearance of the same cercaria in the controls, has invalidated the results. On the other hand, infected snails have been fed to sheep and the eggs of *Dicrocoelium dendriticum* subsequently recovered from the faeces. It has been assumed from these experiments that the normal mode of infection in sheep is obtained by eating infected molluscs while attached to grass.

A study of the anatomy and biology of *Cercaria vitrina* and a consideration of the habitat of all other members of the genus *Dicrocoelium* discloses facts which are not in harmony with this representation of the life history. *Cercaria vitrina* is a stylet cercaria with well-defined salivary glands and although it develops in terrestrial molluscs it possesses a long slender tail. The presence of the first two organs suggests that like all other stylet cercariae it should penetrate and encyst (or develop further) in a second intermediate host, and the presence of a tail that it may be capable of swimming. Vogel (1929) has shown that although the cercaria may live for at least 9 hours in tap water, it does not swim. In all stylet cercariae, however, this is not the only method of locomotion; they are able to progress in a leech-like manner on a surface by means of the oral and ventral suckers, and this method is adopted exclusively when in contact with the second intermediate host. It is therefore highly probable that *C. vitrina* could live on moist or wet grass and soil for some time and by progressing in the manner described would have an opportunity of meeting an insect host. On the other hand, the absence of cystogenous glands would exclude, at first sight, the possibility of encystment, yet these glands are not present in *C. Lecithodendrii chilostomi* which does encyst at a later stage. Vogel attacked the problem in another way. Free *C. vitrina* were incubated at 37° C. in artificial gastric juice and canine intestinal juice; in the former medium they were killed in a few minutes and in the latter they survived for 1 hour. From these experiments it would seem that the cercaria requires some protection, such as a cyst wall, against the action of digestive juices, and the above observations support the view that a second intermediate host is included in the life history.

In addition, all other species of *Dicrocoelium* are usually gall-bladder or bile-duct parasites of insectivorous reptiles, birds and now an insectivorous bat (Macy, 1931). Taking the last case alone, it is clear that there is no possibility of this animal becoming parasitised by eating infected molluscs; its food is

confined to winged insects, one or more of which must serve as second intermediate hosts. In general the life histories of members of the same genus of trematodes, or even related genera, are similar (compare *Gorgodera cygnoides*, *G. varsoviensis* and *Gorgoderina vitelliloba*), the intermediate hosts may be the same species, and it follows that all members of the genus *Dicrocoelium* will have a second intermediate insect host. There is no incompatibility in this statement with regard to the presence of *D. dendriticum* in the sheep, or hosts such as the dog, cat, pig and man among other records given by Nicoll (1923, 1927). Grass harbours a considerable number of winged insects, the foliage, roots and soil may serve as moist habitats for larval stages, and there is probably a greater chance for these herbivorous and carnivorous animals to eat winged insects than terrestrial molluscs. Also grass-eating animals are not necessarily confined to this food, there is usually considerable scope in the vegetarian nature of their diet and incidentally an increased range of winged insect fauna. The presence (although occasional) of *D. dendriticum* in the cat, dog and man also supports the view that there is an insect intermediate host.

In view of these facts I would suggest that the life history of *D. dendriticum* requires a second intermediate host, which is in all probability a dipterous larva, the imago of which is eaten accidentally by the sheep. Since all other species of *Dicrocoelium* are parasites of insectivorous vertebrates, it would appear that the sheep is an unusual host for a member of this genus, and may well be an accidental one.

VII. Summary.

1. The life history of *Lecithodendrium chilostomum* has been established; *C. Lecithodendrii chilostomi* penetrates a second intermediate host, the larva of *Phryganea grandis*, and unlike other stylet cercariae does not encyst, but feeds and grows in the host tissue as a mobile metacercaria. During pupation of the host in the following year these mobile forms migrate from the abdominal segments of the larva to the thorax, where they then encyst in the thoracic muscles in which they are also found in the imago. The largest metacercariae and the excysted worms are typical early adult *Lecithodendrium chilostomum*. No case of progenesis in the metacercaria was found.

2. It is estimated that the maximum swarming of the cercariae probably occurs during July, when first penetration of the intermediate host takes place. Subsequent diminished swarming and penetration proceed until November. The life of the larval trematode as a mobile metacercaria is approximately 8 months; the imagines of *Phryganea grandis* emerge during May and June, the infective period for the final host.

3. The metacercariae are not distributed throughout the body cavity of their larval host, but the majority are confined to the three posterior segments.

4. *Limnophilus rhombicus* may also serve as a second intermediate host, but the infection is very light and the metacercariae do not encyst during pupation of this host.

5. The excretory system has been determined in all stages of *Lecithodendrium chilostomum*; it is of the 2 (6 × 2) type. The occurrence of this type of system in other groups of cercariae is reviewed and since it is found in several widely separated families, it is suggested that its presence does not necessarily imply relationship, but is due to convergence.

6. The life histories of the following bat trematodes are indicated: *Lecithodendrium lagena*, *Plagiorchis vespertilionis* and *Crepidostomum moeticus*.

7. The life history of *Dicrocoelium dendriticum* is discussed.

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EXPLANATION OF PLATE XXI.

All figures are partly diagrammatic and are drawn to different scales.

Fig. 1. *Cercaria Lecithodendrii chilostomi*. Body length 0·16 mm. Reconstructed from early post-cercarial stage, see Fig. 2.

Fig. 2. Early post-cercarial stage from body cavity of larva of *Phryganea grandis*. Anatomy including excretory system. Body length 0·165 mm.

Fig. 3. Metacercaria from body cavity of larva of *P. grandis*. Development of genital system. Body length 0·37 mm. Dorsal view.

Fig. 4. Cyst from thoracic muscles of imago of *P. grandis*. 0·23 × 0·21 mm.

Fig. 5. Young *Lecithodendrium chilostomum* from cyst. General anatomy. Body length 0·6 mm.

Fig. 6. Excretory system of young *L. chilostomum*. Body length 0·6 mm.

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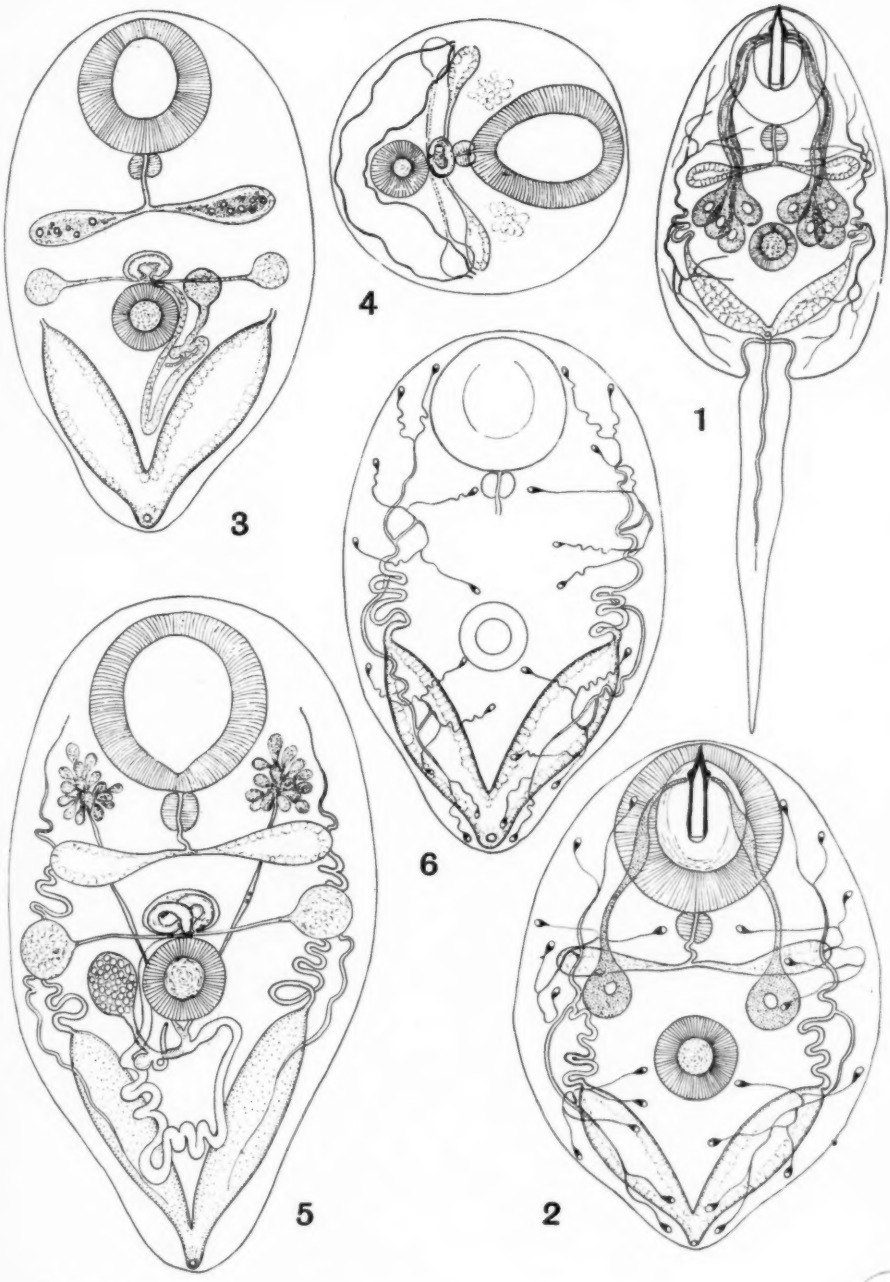


F.J.B. del.

STUDIES ON THE MONOGENEA (TREMATODA) OF PLYMOUTH.

I. *MICROBOTHRIUM CANICULAE* (JOHNSTONE 1911).

By E. IDRIS JONES, B.Sc. (Lond.)

(With 3 Figures in the Text.)

Synonym: *Paracotyle caniculae* Johnstone 1911.

On the skin of the dorsal surface, and particularly on the dorsal fins of *Scyllium canicula* at Plymouth, occurs a parasite which appears to be identical with *Paracotyle caniculae* Johnstone 1911. In British waters it was first noticed by Scott (1906) who queried it as a species of *Epibdella*. Johnstone (1911) gave an account of the species but his specimens were immature, being in the male phase of their development and considerably smaller than those found at Plymouth.

The specimens found at Plymouth are mature and in process of egg-formation. The average length of a specimen is 3–3·2 mm. and 1·5 mm. width. The body is flattened and oval in shape, pointed anteriorly, with an anterior sucker surrounding the mouth and a posterior sucker sub-terminal on the ventral surface, devoid of hooks or rays (Fig. 1). The mouth leads into the vestibule or prepharynx which is followed by the muscular pharynx. The oesophagus is very short and the two limbs of the gut pass down between the vitellaria on either side of the body to terminate a short distance from the posterior end. The limbs of the gut do not give off lateral branches. Paired excretory vesicles open to the exterior on the lateral margins of the body at the level of the oesophagus.

Reproductive system. There is one testis, one ovary and numerous vitellaria. The *testis* is a compact organ situated in the mid-line near the centre of the body. It is transversely oval measuring 0·057 × 0·028 mm. and septa traverse it. From its anterior border a thick, deeply staining vas deferens passes forwards to the cirrus sac. The *ovary* is spherical, situated immediately anterior to the testis, measuring 0·032 mm. in diameter. The oviduct leads from it anteriorly and receives the common vitelline duct, after which it leads directly into the shell gland. From the shell gland the uterus emerges and passes ventral to the cirrus sac to the common genital opening which is situated in the mid-line posterior to the oesophagus. The *vitellaria* are compact bodies arranged on either side of the limbs of the gut but none are situated posterior to its termination. The *cirrus sac* has very muscular walls and contains the pars prostatica, ductus ejaculatorius and the chitinous cirrus (Fig. 2). The ductus ejaculatorius is relatively very much smaller than in Johnstone's specimens. On the left of

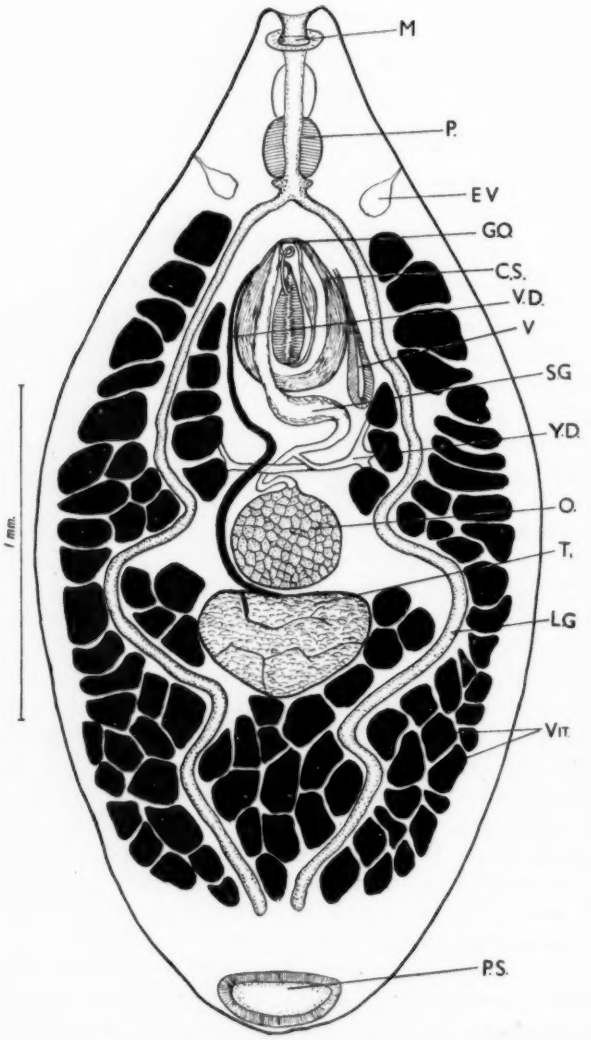
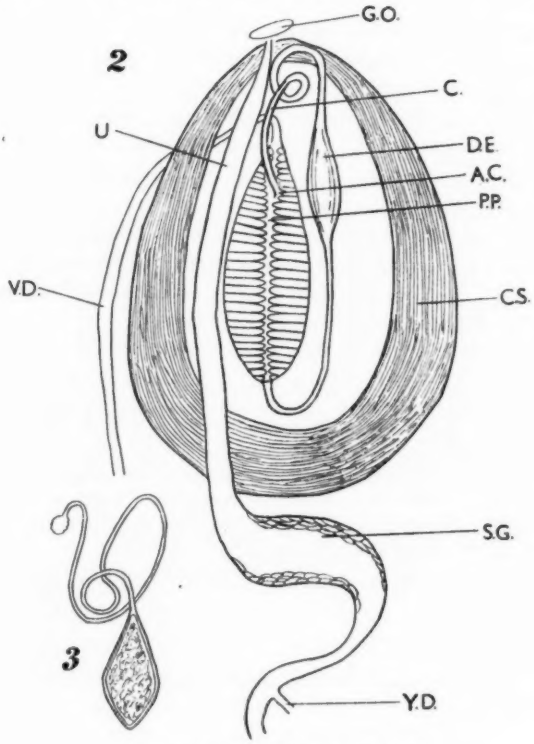


Fig. 1. *Microbothrium caniculae.* Entire animal. Ventral view.

Lettering: *A.C.* aperture of cirrus; *C.* cirrus; *C.S.* cirrus sac; *D.E.* ductus ejaculatorius; *E.V.* excretory vesicle; *G.O.* genital opening; *L.G.* limb of gut; *M.* mouth; *O.* ovary; *P.* pharynx; *P.P.* pars prostatica; *P.S.* posterior sucker; *S.G.* shell gland; *T.* testis; *U.* uterus; *V.* vagina; *V.D.* vas deferens; *Vit.* vitellaria; *Y.D.* yolk duct.

the cirrus sac is the vagina which has muscular walls but the connections with the rest of the genitalia cannot be made out. It may be a vestigial structure as Johnstone suggests.

The *eggs* (Fig. 3) are large and only one is observed in each specimen. They are oval in shape, pointed and provided with a long filament at the opercular pole. Each egg measures 0·0144 mm. in length.



Figs. 2, 3. *Microbothrium caniculae.*
Fig. 2. Cirrus sac and associated structures.
Fig. 3. Egg.
For lettering see Fig. 1.

The specimens here described differ from Johnstone's in their size, his specimens being 1–2 mm. long, and in the fact that they are mature.

In looking for the relationships of the species Johnstone says: "There is only one testis in this species and this causes it to occupy an isolated position among Tristomidae and Monocotylidae. . . . Of the genera of the Monocotylidae only *Pseudocotyle* has a posterior sucker devoid of rays and hooks, but it has numerous testes and a branching gut." The nearest known genus he suggests is *Monocotyle* which has a small lateral unpaired vagina on the left, but has three

testes closely apposed and ridges and hooks on the posterior sucker. Olsson (1893) described *Dactylodiscus borealis* Olsson from the gills of *Thymallus vulgaris*. This species has a single testis and unpaired vagina but has four eyes and hooks and finger-like processes posteriorly. Olsson (1869) founded a new genus, *Microbothrium*, for small trematodes which he found on the skin of Elasmobranchs. As a generic diagnosis he gives: "*Microbothrium* n.g. Corpus planum, postico bothrio longitudinali lanceolato, inermi. Crura intestini ramos extorsum emittentia." His description of species does not include a form from *Scyllium canicula*, but there seems little doubt that Johnstone's *Paracotyle* is a genotype of Olsson's *Microbothrium*. *Paracotyle caniculae* Johnstone has an unbranched gut and larger vitellaria, but otherwise it is identical with *Microbothrium apiculatum* Olsson. These differences are of specific value only and thus the form here described should be called *Microbothrium caniculae* (Johnstone 1911).

Its nearest relatives occurring at Plymouth are *Calicotyle krøyeri* Diesing in the cloaca of *Raia* spp. and *Pseudocotyle squatinae* v.Ben. and Hesse on the skin of *Rhina squatina*. It has not before been recorded from Plymouth and this is the third record of its occurrence in British waters.

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FOUR NEW SPECIES OF AVIAN CESTODES FROM INDIA.

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(With 14 Figures in the Text.)

The present paper deals mainly with some new species of cestodes collected from birds commonly occurring in and near Nagpur, C.P., India.

Baeria orbiuterina gen. et sp.nov.

(Figs. 1–4.)

Specimens of this worm were obtained from the intestine and gizzard of *Turdoides somervillei* Sykes.

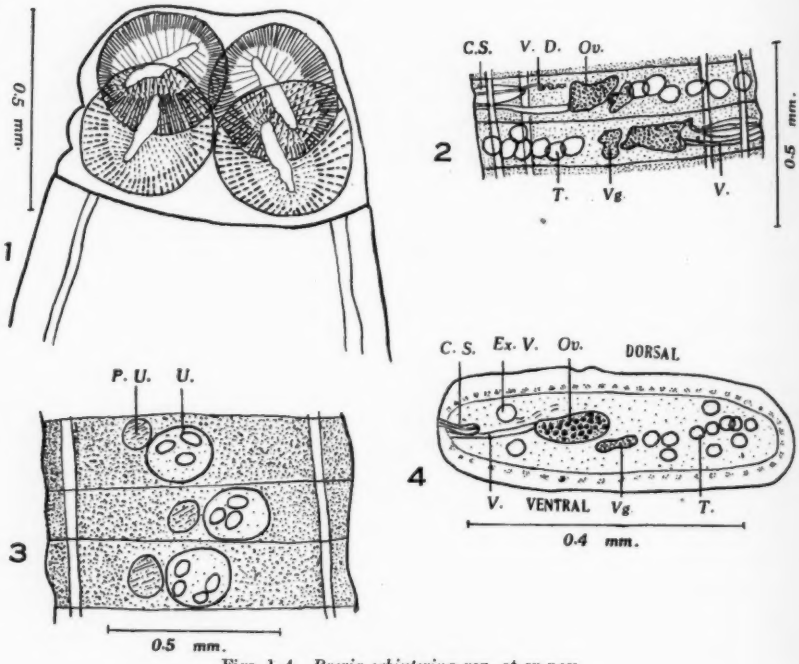
Anatomy. Maximum length of worm about 35–40 mm. Maximum width anteriorly 0·9 mm.; in some specimens, the scolex is the widest part of the worm; the width is, more or less, uniform, slightly narrowing posteriorly. The anterior proglottids (containing ovary, but no testes or genital ducts) measure 0·12 × 0·715 mm.; mature proglottids 0·12 × 0·685 mm.; gravid ones 0·225 × 0·675 mm.; and the terminal proglottid in one specimen 0·50 × 0·55 mm. All segments are wider than long.

The maximum width of scolex (Fig. 1) 0·923–1·5 mm. Diameter of large globular suckers 0·375–0·562 mm. Rostellum and "neck" absent, but a distinct segmented portion of strobilus behind scolex (2·58 mm. long) which contains no reproductive organs or ducts. Genital pores irregularly alternating; genital ducts passing between excretory vessels.

Genital pores situate midway on lateral margin of proglottid. Genital atrium very shallow. Cirrus sac (Figs. 2 and 4, *C.S.*) anterior to vaginal canal and 0·11 mm. long. Cirrus in the sac a straight tube continuous with loosely coiled vas deferens (*V.D.*). Ovary (*Ov.*) slightly poral measuring in fully mature proglottids 0·16 × 0·063 mm. Vaginal canal at junction with ovary forms a slightly dilated receptaculum seminis. Vitelline gland (*Vg.*) lies partly in the same line beyond and posterior to ovary. In same line, on aporal side of ovary, there are about 8–9 testes (*T.*), each 0·044 mm. in diameter, extending beyond excretory vessels and nearly attaining lateral margin of proglottid. Segments, beyond a third or slightly more of entire strobilus, have uterus (Fig. 3, *U.*) appearing as median sac-like globular structure occupying nearly whole length of proglottid. Uterus with spherical parauterine organ

(*P.U.*). Eggs thin shelled, without pyriform apparatus and measuring (in balsam) 0·023 × 0·019 mm.

Systematics. This worm, having no rostellum and all proglottids being wider than long, belongs to the family Anoplocephalidae and subfamily Anoplocephalinae. This subfamily contains 20 genera: 17 listed by Baer (1927), *Pseudoanoplocephala* Baylis 1927, *Killegrewia* Meggitt 1927, and *Taufikia*



Figs. 1–4. *Baeria orbiuterina* gen. et sp.nov.

Fig. 1. Scolex.
Fig. 2. Entire mature proglottids.
Fig. 3. Entire gravid proglottids.
Fig. 4. Transverse section through a mature proglottid.

Lettering: *C.S.* cirrus sac; *Ex.V.* excretory vessel; *G.A.* genital atrium; *I.V.S.* internal vesicula seminalis; *Ov.* ovary; *P.U.* parauterine organ; *R.S.* receptaculum seminis; *T.* testes; *U.* uterus; *V.* vagina; *V.D.* vas deferens; *Vg.* vitelline gland.

Woodland 1928. Of these 20 genera, those possessing a single set of genital organs, with parauterine organ present and having irregularly alternating genital pores, are seven as follows, viz. *Bertiella* Stiles and Hassall 1902, *Parabertiella* Nybelin 1917, *Paranoplocephala* Lühe 1910, *Prototaenia* Baer 1927, *Schizotaenia* Janicki 1914, *Killegrewia* Meggitt 1927 and *Taufikia* Woodland 1928. Of these genera, three, viz. *Bertiella*, *Parabertiella* and *Taufikia*, have testes anterior to ovary; *Prototaenia* have pedunculated suckers and an

armed cirrus; in *Schizotaenia* the vagina lies in front of cirrus sac; in *Killegrewia* the testes are in two equal groups. Therefore the worm herein described does not belong to any of these genera.

There remains but one genus to be considered, *Paranoplocephala* Lühe 1910, with characters resembling those of the worm herein described, *e.g.* in having genital pores irregularly alternating (though some species of *Paranoplocephala* have unilateral genital pores) and testes placed as described. But in *Paranoplocephala* Lühe, the uterus is spherical and lobed, the eggs have a pyriform apparatus and the genital ducts are dorsal to excretory vessels. These characters are sufficiently different from the characters of the worm herein described to justify the creation of a new genus for which I propose the name *Baeria* and designate *B. orbiuterina* as the type species.

Diagnosis of the genus. Anoplocephalinae. Genital pores irregularly alternating; genital ducts pass between excretory vessels; a single set of reproductive organs in each segment; testes situated beyond the ovary on its poral side; uterus a globular sac with a parauterine organ; eggs without pyriform apparatus. Adults in birds.

Diagnosis of species. Strobilus about 35–40 mm. long; maximum width 0·9–1·25 mm. Scolex large, 0·923 mm. long and about 1·5 mm. wide with large globular suckers 0·375–0·562 mm. in diameter. Testes numbering about nine, situated beyond the ovary on its poral side, each 0·044 mm. in diameter; cirrus sac small, 0·11 mm. long; ovary poral; eggs (in balsam) 0·023 × 0·019 mm.

Host. Turdoides somervillei Sykes.

Habitat. Intestine and gizzard.

Locality. Nagpur, C.P., India.

Ophryocotyloides meggitti sp.nov.

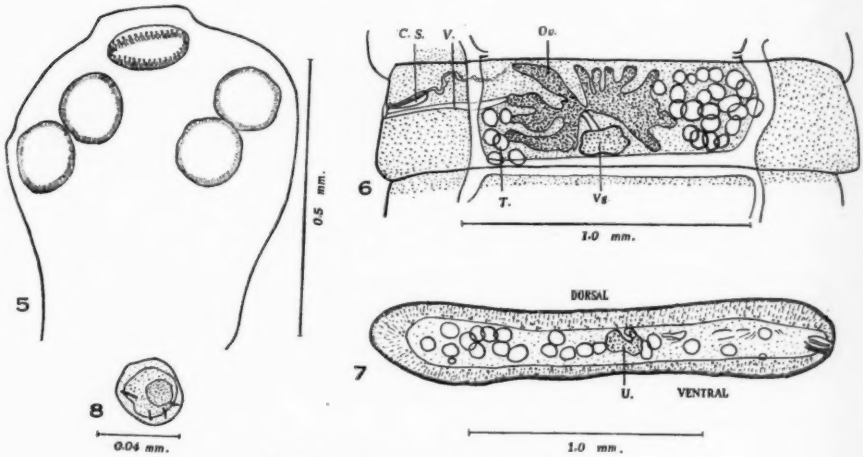
(Figs. 5–8.)

I obtained about six specimens of this cestode from the intestines of house crows, *Corvus splendens* L., in which infestation is general.

Anatomy. Maximum length of the worm 330 mm. Maximum width 3 mm. Scolex (Fig. 5) 0·6 mm. long with maximum width 582 μ. Rostellum 93 × 153 μ, armed with a double row of many small hammer-shaped hooks of Davaineid type. Hooks 16 μ and 14 μ long. Suckers more or less globular, each sucker 133 μ in diameter and armed with minute hooks. Anterior proglottids (*i.e.* those in which only the ovary is present as a strand of tissue) 0·194 × 1 mm. Proglottids with all genital organs developed but ovary not of maximum size 0·305 × 2 mm. Fully mature proglottids 0·347 × 2 mm.; gravid segments 1·56–2·8 × 2·4–3 mm.

Genital organs in mature segments disposed as follows: Testes (Fig. 6, *T.*) number about 40, of which 35–36 on aporal and 5–6 on poral side of ovary do not extend beyond excretory vessels and lie entirely lateral to ovary. Each testis about 63 μ in diameter. Cirrus sac (*C.S.*) pyriform or cylindrical,

with inner end halfway between lateral margin of proglottid and excretory vessel of that side, 136 × 15μ and contains a nearly straight unarmed cirrus. Vas deferens (*V.D.*), seen in transverse section, disposed in few simple coils and dorsal to excretory vessels. Genital atrium very shallow, genital pore situated anterior to middle point of lateral margin of proglottid. Genital pores unilateral. Vagina (*V.*) posterior to cirrus sac and passing close to it as a straight tube till it attains middle of ovary. Ovary (*Ov.*) in fully mature proglottid much branched, occupying nearly whole length of proglottid and nearly a third of its width. Vitelline gland (*Vg.*) very conspicuous, posterior and slightly lateral to ovary. Uterus, in early gravid segments, a narrow bent



Figs. 5–8. *Ophryocotyloides meggitti* sp.nov.

Fig. 5. Scolex.
Fig. 6. Entire mature proglottid.
Fig. 7. Transverse section through a mature proglottid.
Fig. 8. Egg (only four hooks could be seen).

(For lettering see Figs. 1–4.)

tube (Fig. 7, *U.*) in centre of proglottid, but when fully formed, a wide sac occupying nearly whole proglottid and extending beyond excretory vessels: it persists and does not break into egg capsules. Eggs (Fig. 8) 30 × 29μ (in balsam).

Excretory system consists of two pairs of canals, the ventral pair being joined by a transverse canal at posterior border of segment.

Systematics. This cyclophyllidean cestode belongs to the family Davaineidae Fuhrmann 1907 since it has a rostellum armed with many hammer-shaped hooks. The Davaineidae include three subfamilies of which only the Ophryocotyliinae Fuhrmann 1900 possesses a persistent uterus. The species herein described consequently belongs to this subfamily and to the genus *Ophryo-*

cotyloides Fuhrmann 1920, because the genital pores are unilateral in contrast to the only other genus in the subfamily *Ophryocotyle* Friis 1870, in which they are alternate. Only two species of this genus have so far been described: *O. uniuterina* (Fuhrm. 1909), first described as *Davainea uniuterina* Fuhrm. 1909, and *O. pinguis* (Fuhrm. 1904), originally described as *Bertia pinguis* (1904). Both were subsequently placed in the genus *Ophryocotyloides* (Fuhrmann 1921). Table I gives the characters of the species of this genus.

Table I. *Characters of species of* Ophryocotyloides.

(Measurements in mm.)

	O. uniuterina (Fuhrm. 1909)	*O. pinguis* (Fuhrm. 1904)	*O. meggitti* sp.nov.
Length	60	70	330
Width (maximum)	0·8	4	2·4–3
Scolex (diameter)	0·5–0·4	0·6	0·6
Suckers (diameter)	0·14	0·22 × 0·26	0·133
Rostellum (diameter)	0·022	1	0·153 × 0·093
Hooks (number)	250	150–200	Many, nearly 200
Hooks (length)	0·018	0·005–0·007	0·016 and 0·014
Testes (number)	50–60	100	40
Cirrus sac (dimensions)	0·22 × 0·06	0·68 × 0·04	0·136 × 0·015
Eggs	?	?	0·030 × 0·029
Host	*Rupicola rupicola* L.	*Bucorax abyssinicus* (Bodd)	*Corvus splendens* Vieill.
Distribution	South America	Africa	India

It is evident from Table I that the species described herein differs from the other two. Therefore, I propose for it the name *O. meggitti*, in honour of Dr F. J. Meggitt who rendered me valuable help in working out this species.

Specific diagnosis. Maximum length 330 mm.; maximum width 3 mm.; scolex 0·6 × 0·582 mm.; rostellum 153 × 93 μ; hooks on rostellum 16 and 14 μ long; suckers armed, 0·133 mm. in diameter; testes 40 in number of which 5–6 are poral; diameter of testis 63 μ; cirrus sac 136 × 15 μ; ovary large; eggs (in balsam) 30 × 29 μ.

Host. House crow, *Corvus splendens* Vieill.

Habitat. Intestine.

Locality. Nagpur, C.P., India.

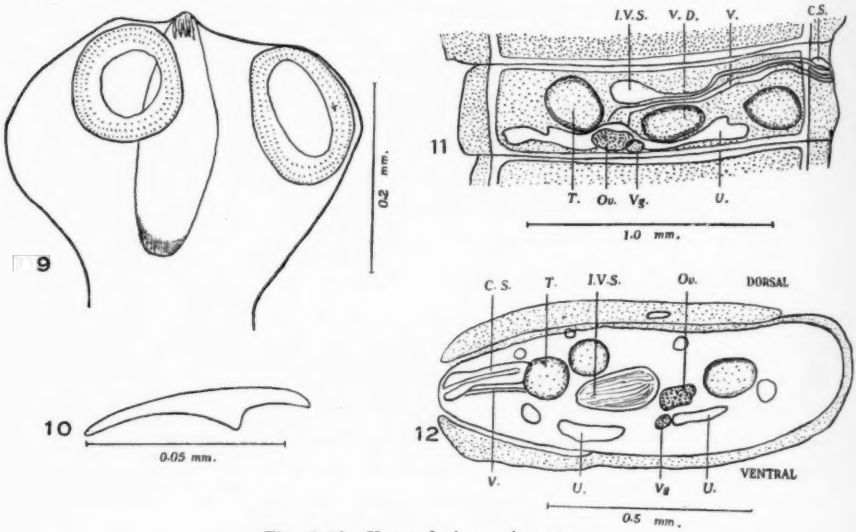
Hymenolepis oweni sp.nov.

(Figs. 9–12.)

I obtained specimens of this worm in pieces from the intestine of *Philomachus pugnax* L.

Anatomy. Maximum length approximately 25–50 mm. Anterior segments without sex organs; first sex organ to appear is ovary, then appear genital ducts and lastly testes. All segments wider than long; proglottids with ovary, testes and ducts, but in which vesicula seminalis is not of the same size as in a mature proglottid, 0·212 × 0·9 mm.; fully mature proglottids 0·18 × 1·2 mm.; and gravid segments 0·25 × 1·5 mm. Maximum width of scolex (Fig. 9) 0·368 mm. Rostellum with its sac 0·25 mm. long and 0·056 mm. in

maximum width. The sac reaches beyond the posterior borders of suckers. Rostellum armed with 10 hooks, each 50–58 μ long, and has the shape shown in Fig. 10. Suckers nearly globular, 0·15 × 0·13 mm.; armed with very minute hooks like those borne on suckers of species of the subgenus *Echinocotyle*. The arrangement of genital organs in a mature proglottid shows the usual Hymenolepid plan. Genital pores unilateral and situate in the anterior fourth of the lateral proglottid margin. Cirrus sac and vagina both open in a genital atrium. Male genital duct is in three different parts: (1) a distal pyriform sac, the outer cirrus sac (Fig. 11, *C.S.*), 0·174 mm. long and 0·053 mm.



Figs. 9–12. *Hymenolepis oweni* sp.nov.

Fig. 9. Scolex.
Fig. 10. Rostellar hook.
Fig. 11. Entire mature proglottid.
Fig. 12. Transverse section through a mature proglottid.

(For lettering see Figs. 1–4.)

in diameter at its base, containing a rod-shaped unarmed cirrus; (2) this is continuous with vas deferens (*V.D.*) which in the middle of the proglottid is dilated to form (3) a vesicula seminalis (*I.V.S.*), 0·294 mm. long and 0·134 mm. in diameter. There are three testes in each proglottid: two poral and one aporal. This arrangement is constant in all segments. Each testis is 0·25–0·312 × 0·137–0·175 mm. Ovary (*Ov.*), in immature segments, appears as a strand of tissue in posterior region of segment between median and aporal testes. In mature segments, it is about 200 μ wide. Vitelline gland (*Vg.*) lies posterior and lateral to and in close contact with ovary. Vaginal canal a narrow tube for the greater part of its length. It expands into a receptaculum

seminis very near its junction with ovary. Uterus (*U.*) commences to appear as a narrow elongated tube at the same position as the ovary but ventral to it; in more gravid segments it fills the whole proglottid. Diameter of the spherical egg (in balsam) is about 80 μ.

Systematics. The possession of a scolex armed with a single row of hooks, segments wider than long, unilateral genital pores, and the position of the genital ducts (dorsal to excretory vessels), three testes in each proglottid and the presence of an external as well as an internal vesicula seminalis, place this worm in the genus *Hymenolepis* Weinland 1858. Referring to the key to species of this genus recorded from India (Southwell, 1930, p. 118), the species with ten hooks are *H. zosteropis* Fuhrm. 1918, *H. annandalei* Southwell 1931, *H. spinosa* v. Linstow 1906, *H. farciminosa* (Goeze 1782), and *H. fusa* (Krabbe 1869) Fuhrm. 1906. The species described in this paper differs from all these in having longer rostellar hooks. But to confirm the validity of our species, I have carefully compared it with the descriptions of all species of *Hymenolepis* possessing ten hooks on the rostellum (*vide* list by Fuhrmann, 1924, and other species since recorded).

Specific diagnosis. Length 25–50 mm.; maximum width 1·5 mm.; diameter of scolex 0·368 mm.; rostellum armed with ten hooks, each 50–58 μ long; length of rostellum 0·25 mm. and its width 0·056 mm.; suckers armed with minute spines; cirrus sac 0·174 × 0·053 mm.; two poral and one aporal testes, each 0·25–0·312 × 0·137–0·175 mm.; eggs (in balsam) 80 μ in diameter.

Host. Philomachus pugnax L.

Habitat. Intestine.

Locality. Nagpur, C.P., India.

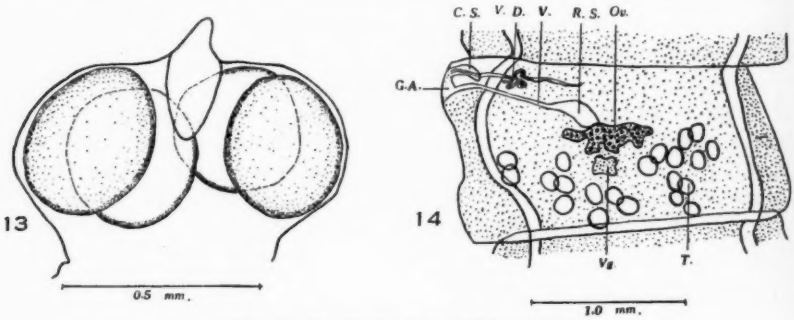
Unciunia acapillicirrosa sp.nov.

(Figs. 13, 14.)

Five specimens were collected from the small intestine of one common duck, *Anas platyrhyncha* (*domestica*) Linn. These belong to the genus *Unciunia* Skrjabin 1914. So far only two species of this genus have been recorded: *U. trichocirrosa* Skrjabin 1914 and *U. sudanea* Woodland 1928, from widely different avian hosts and from different zoogeographical regions. The species described herein will be the third species of this genus and the only one from India.

Anatomy. Length 90–110 mm.; maximum width 1·17 mm. Proglottids all wider than long; anterior ones (*i.e.* without any sex organs) 0·31–0·34 × 0·97 mm.; those in which the genitalia are formed but are not fully mature 0·3 × 0·93 mm.; mature ones 0·83 × 0·93 mm. and gravid ones 0·83 × 1·17 mm. Posterior proglottids are more or less quadrate in shape. Scolex (Fig. 13), 0·462 × 0·846 mm., bears four globular suckers, each 0·4 mm. in diameter and a distinct unarmed rostellum measuring 0·31 × 0·13 mm.

There is an unsegmented "neck" region 0·454 mm. in length. Genital pores irregularly alternating and the genital ducts pass between the excretory vessels. A well developed genital atrium is present. Cirrus sac (Fig. 14, *C.S.*), 0·19 × 0·057 mm., does not reach the excretory vessel of the opposite side as in *U. sudanea* Woodland 1928 and has no hairs or spines at its base as in *U. trichocirrosa* Skrjabin 1914. It contains a straight cirrus which at its inner end forms a much coiled vas deferens (*V.D.*). Testes (*T.*) are approximately 60 in number, situated posterior and lateral to ovary, each 0·061 × 0·048 mm. Vagina (*V.*) a simple tube opening into genital atrium posterior to cirrus sac, expands near the ovary into a club-shaped receptaculum seminis (*R.S.*). No gland cells were observed round about the vagina. Ovary (*Ov.*), a transversely elongate structure in a mature proglottid, measures 0·58 mm. in width. Vitelline gland (*Vg.*) posterior to ovary. Uterus, in gravid segments, is sac-like and divided by strands of tissue into compartments each containing several eggs. Eggs are thin-shelled and (in balsam) are 0·015 × 0·012 mm.



Figs. 13, 14. *Unciunia acapillicirrosa* sp.nov.

Fig. 13. Scolex.

Fig. 14. Entire mature proglottid.

(For lettering see Figs. 1–4.)

Systematics. This worm evidently belongs to the family Hymenolepididae and to the subfamily Dilepininae Fuhrm. 1907. It belongs to the genus *Unciunia* Skrjabin 1914, since it is the only genus with an unarmed rostellum in this subfamily. It differs from *U. sudanea* Woodland 1928 in having a distinct rostellum and in having a much shorter cirrus sac and in the absence of gland cells round the vagina. It differs from *U. trichocirrosa* Skrjabin 1914 in having a larger number of testes and in the absence of hairs or spines at the base of the cirrus sac. These differences are sufficiently well marked to justify making a new species for this worm for which I propose the name *Unciunia acapillicirrosa.*

Specific diagnosis. Length 90–110 mm.; maximum width 1·17 mm.; scolex 0·461 × 0·846 mm.; a small but distinct rostellum present; suckers 0·4 mm. in diameter; testes 60 in number posterior and lateral to ovary; cirrus sac

0·19 mm. long; receptaculum seminis club-shaped; eggs (in balsam) 0·015 × 0·012 mm.

Host. *Anas platyrhyncha* (*domestica*) Linn.

Habitat. Small intestine.

Locality. Nagpur, C.P., India.

NEW RECORDS OF KNOWN SPECIES OF CESTODES.

Host: *Turtur cambayensis.*

Cotugnia fuhrmanni Baczynska 1914, previously recorded from *Pavo cristatus* from Ceylon.

Hymenolepis serrata Fuhrm. 1906, not yet recorded from India.

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THE LIFE HISTORY OF *ONESIA ACCEPTA* MALLOCH (DIPTERA, CALLIPHORIDAE).

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(With Plate XXII, containing Figs. 1–5, and Figs. I–X in the Text.)

I. Introduction.

The first record of the early stages of *Onesia* was published in 1915 by Keilin, who obtained first stage larvae of *Onesia sepulchralis* Meig. from earthworms. Thompson in 1921 dissected the young larvae from a female fly of the same species and described them in detail. Neither of these workers succeeded in obtaining the complete life history of this species. Keilin, however, presumed that it would be similar to that of *Pollenia rudis*, whose complete life history as a parasite in earthworms he traced.

II. Systematics.

The fly on which the present study has been carried out agrees with the definitions of Bezzi (1927), Séguy (1928) and others of the genus *Onesia*, although there is some controversy amongst the various authors as to the exact status of the genus, Malloch (1932) preferring to include it under *Calliphora*. But, apart from the systematics of the adult, this species has been found closely to resemble *O. sepulchralis*, as described by Keilin, in larval characters and habits. Therefore it has been decided to adopt the name *Onesia* in the present paper.

The species was described recently by Malloch (1927) as *Calliphora accepta*. My specimens agree with his description and the adults will not be described again here.

III. Observations on life history and habits.

Onesia accepta first came under my notice among the catch in the blowfly trap at the laboratory in November, 1931. It could not be induced to breed, and on dissection showed the ovaries to be undeveloped. In April, September and October, 1932, this species was again present in the trap.

During the second week of September, 1932, the fly was discovered in greater abundance than ever before, frequenting a hedge of *Photinia serrulata* (Chinese hawthorn, an introduced plant) and hovering in the long grass adjacent on a vacant allotment in a suburb of Canberra. The flies were sluggish in their movements and when disturbed from the hedge soon returned. Many

were feeding from aphides (*Myzius persicae* Sulz.) which were thick on the younger shoots.

In rain and mist the flies were more active than in the sunshine, leaving the hedge and crawling or flying over the grass close to the ground. The flies were abundant in this locality for three weeks, but by the middle of October very few remained. During the same period they were common in the trap.

Evidence pointed to *Onesia* being parasitic, so the grass and soil where the adults were so abundant were searched for possible hosts. Slugs, caterpillars and beetle larvae were all common, but earthworms were the most plentiful. Although a search was made each week in the soil, it was not until a month after the flies had first been noticed, and when they had become scarce, that earthworms containing maggots were found.

On October 17th a number of worms were dug up and when they were washed clean of soil it was found that a fair percentage were parasitised. These worms had a characteristic appearance, being easily distinguished from the normal ones (Pl. Fig. 1). They were constricted in places giving them an uneven or twisted shape (Fig. 2). The constrictions were due to the tracks made by maggots crawling under the skin, the maggot travelling round the worm in a spiral path and moving towards either end. The track is noticeable as a white mark where the body has contracted away from the skin, the young maggot being invariably found at one end. In all the worms taken in the field there was only one maggot, with a single exception in which there were two (Fig. 3).

The worms were sent to the Australian Museum for identification and proved to be *Microscolex dubius* Fletcher, an introduced species. Since there is no reason to doubt Malloch's determination of the fly as an Australian species, the original host, as yet undiscovered, must also have been native.

First and second instar maggots were found under the skin of *Microscolex*.

A week later a further collection was made in the same locality. Adult flies were now very scarce. As in those collected earlier, the worms also had first or second instar maggots under the skin, but in addition some had second and a few had third instar larvae inside the body cavity, just visible from the outside. Two of the worms had third stage maggots projecting from the end (Fig. 5).

Finally, on November 19th, the area was again examined. All stages of the larval *Onesia* were found in worms, including very young maggots. No puparia were found, but from some soil collected on the previous occasion a fly emerged so that a prepupa or puparium must have been present in it.

IV. METHOD OF BREEDING THE FLY.

In September, some hundreds of *O. accepta* were collected in the grass and on the hedge and kept in cages. On dissection many of the females were found to contain numerous small live maggots. Some of these were placed on various

media, such as liver, crushed earthworm and yeast, but failed to develop, although they remained alive four days.

In March and April, 1930, a closely related species, *Onesia australica* Mall., was taken in large numbers in the hills about 30 miles from Canberra. This was flying low in the native grasses in the same manner as *O. accepta*. It was found to be larviparous, but was not bred, although it is undoubtedly a parasite of some native worm.

Petri dishes containing slugs, worms and caterpillars were placed in the cages with *O. accepta*. The flies deposited larvae on the soil in dishes containing worms and slugs, and young maggots were found crawling on the surface of these animals and congregated underneath them in the slime. Later, first instar maggots identical with those taken from the female flies were found under the skin of earthworms in the cages, but none were found inside slugs. Second stage maggots were also found in worms and in the soil, and finally the third instar larvae were found feeding in living worms and in dead rotting ones. None developed in slugs. The maggots pupated in the soil.

Living worm tissue is essential to the first and second stage larvae, but in the third stage the maggot is capable of feeding on dead and even putrid worms. In the field the maggots were found only in *Microscolex dubius*, but in the cages they thrive also in *Allolobophora caliginosa* Sav. subsp. *trapezoides* Ant. Dug. The former species lives in the soil and among grass roots. *Allolobophora* was dug up in large numbers in the vicinity of a manure heap, and was not found to be infected with *Onesia*.

The habit of the adults of flying near the ground and in the grass in wet weather is doubtless associated with the fact that the worms are on or near the surface and the young larvae deposited then have a chance of finding and entering their hosts.

During September, when the soil and grass are constantly moistened by dew and fogs, the flies are numerous, and a few weeks later, when the soil has hardened and the grass dried up, the flies disappear.

Although the flies come to carrion baited traps they never deposit larvae there. Many females were dissected at the same time as those found in the grass and none contained maggots. They doubtless come to the carrion to obtain protein food which is essential for the development of the ovaries (Cousin, 1929; Mackerras, 1932).

On dissecting and counting the contents of the ovaries of one female *Onesia*, 550 larvae were found in various stages of development. This fecundity is characteristic of a parasitic insect with such habits as *Onesia*. Another parasitic adaptation is the ability of the first stage larvae to exist for some days before beginning to feed and grow. The young maggots of other blowflies quickly perish if the proper food is not immediately available.

V. LENGTH OF STAGES IN LIFE CYCLE.

The following data were obtained from the first lot of maggots deposited in the cages. The young maggots were seen on the soil and under the skin of the worms on September 30th. The first fly emerged on November 7th. As far as could be determined the stages occupy the following times. The first instar occupies 4 or 5 days, being passed under the skin of the worm. The second instar takes about 4 days and is also passed under the worm's skin. The maggot may become free at the end of the second instar and crawl about on the surface of the worm where it feeds, but generally it enters the body of the worm and passes the third stage there. This occupies 11 days. Thus the whole feeding period is about 20 days. During this time the worm may remain active and intact and large third stage maggots have been seen protruding from worms which seem to be quite active. In many cases in the cages the worms disintegrated and rotted and the third instar maggot fed readily on the dead material, although earlier stages apparently cannot do so. Often the worms infected with large maggots were broken at the clitellum. It has not been determined in what manner the maggot penetrates the skin of the worm, as the tracks appear to start at any place, nor has it been discovered how the third stage maggot enters the body cavity of its host, as it is found in any part of the worm.

When full grown, the maggot burrows into the soil, leaving what remains of the worm, which soon dies. It remains prepupal 3 or 4 days. The pupal period occupies 12 days.

Maggots reared somewhat later during October and November passed through the various stages in a slightly shorter period than the first batch. The feeding stage was never less than 17 days and the pupal stage never less than 10.

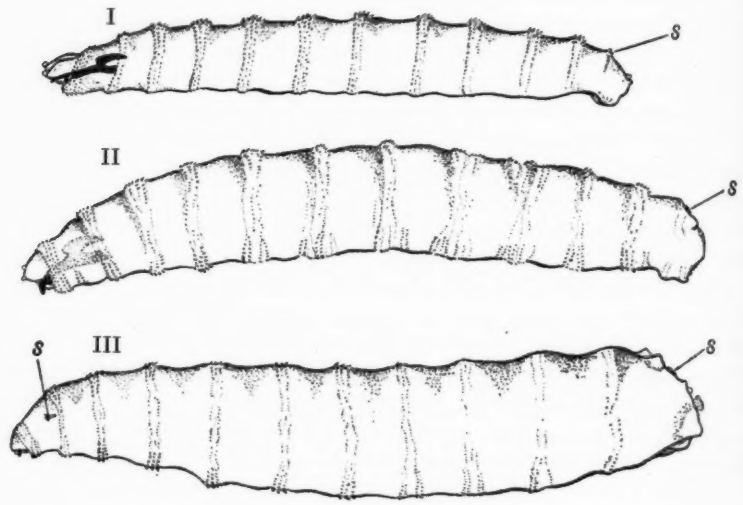
VI. DESCRIPTION OF THE EARLY STAGES.

First stage larva (Fig. I).

The average length of those taken from worms is 1·6 mm. Maggots removed from the ovaries of female flies are slightly smaller. The maggot is cylindrical in shape, with a pointed head and truncated posteriorly. Behind the head there are three thoracic, eight ordinary and two reduced abdominal segments. The head is divided into two lobes pointed anteriorly, each bearing an antenna and a maxillary palp. The antenna is comparatively large and is a single dome-shaped segment situated near the front of the head lobe dorsally. The maxillary palp is below the antenna and slightly anterior to it. It is a flat disc with three small papillae arising from it.

The skin is thin and very transparent so that the whole of the buccopharyngeal armature is clearly visible. This is very elongated and may extend back to the beginning of the third thoracic segment. The anterior third of the

first thoracic segment is thickly covered with spines which are directed forwards in the first one or two rows and the rest backwards. The anterior border of the second and third thoracic segments is also covered with spines which are most strongly developed dorsally and are directed backwards. The abdominal segments are marked off by strong annulations and from the first to the seventh bear a tuft of large spines on the dorsal surface which are distinctly curved backwards. These are lightly chitinised and semi-transparent. The posterior spiracles appear on the eighth segment as a pair of tubes diverging outwards. They open on the surface as a single slit with an inward projection at one side. There are no anterior spiracles.



Figs. I–III. *Onesia accepta.*

Fig. I. First stage larva. ×53.
Fig. II. Second stage larva. ×43.
Fig. III. Third stage larva. ×9; *s.* spiracles.

Mouth-parts (Fig. IV). The armature is composed of two parts, a basal or pharyngeal sclerite and a buccal sclerite. The pharyngeal sclerite is divided at the posterior end into the usual dorsal and ventral cornua. Anteriorly it is elongated and divided into two branches. Keilin found a similar condition in the first stage larva of *Onesia sepulchralis*. The buccal sclerite consists of two large hooks which are narrow, pointed and slightly curved ventrally. These articulate with the anterior branches of the pharyngeal sclerite. Towards the base of the hooks and ventrally there is a pair of small triangular sclerites.

Second stage larva (Fig. II).

The change to second instar takes place at an early stage and while the maggot is still very small. Second stage larvae taken from under the skin of a worm measured usually 2·5 mm.

The first thoracic segment is constricted in the middle and very spiny on the anterior half. The anterior spiracles are small and project laterally from the posterior border of the first thoracic segment. All the body segments are marked off anteriorly by bands of dark brown spines, usually five rows to each band. These are not in large dorsal tufts as in the first stage, but are equally well developed dorsally and ventrally, fading out on the sides. Abdominal segments 4–7 have also a few rows of spines on the posterior border dorsally and ventrally making a double band in these parts. Segment 8 is of the usual shape and has a long sloping antero-dorsal surface with some rows of spines round its edge. The spiracles are near the upper edge of this surface, being slightly raised with two slits in each plate. Segment 9 is entirely ventral, below and a little behind the eighth, and consists of a large rounded protuberance covered with minute spines. The anus occurs in a fold surrounded by rows of slightly larger spines on the anterior edge of the ninth segment.

Mouth-parts (Fig. V). The buccopharyngeal armature consists of three parts. The pharyngeal sclerite has the usual dorsal and ventral cornua posteriorly, and anteriorly it articulates with the intermediate or hypopharyngeal sclerite. At the anterior edge of the pharyngeal sclerite a pair of narrow chitinous rods project forwards over the middle sclerite dorsally. The hypopharyngeal sclerite is small and closely connected with the basal sclerite. It has a blunt ventral projection and a narrow curved rod-like sclerite running forwards to the base of the buccal hooks ventrally. The buccal hooks are comparatively shorter and broader than in the previous stage. They have a concavity at the base where they meet the middle sclerite and have a ventral projection in the middle. They are strongly curved and sharp. Near the ventral projection are two small sclerites.

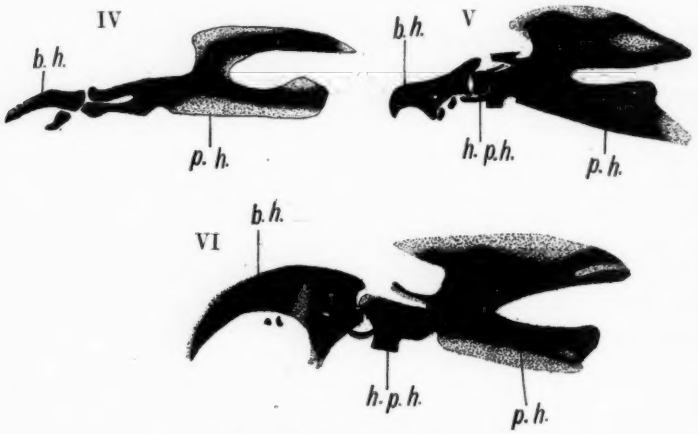
Third stage larva (Fig. III).

The final moult also takes place when the maggot is very small, and third stage maggots measuring 4 mm. have been dissected from worms. The size of the maggot at the end of the feeding stage varies considerably. The full-grown maggot, on which the following description is based, measured 11 mm., but much smaller and larger maggots have been seen to leave the worms and pupate. The colour is creamy white. The maggot has the usual Calliphorid form, being pointed anteriorly and broadly truncated posteriorly. The head is very small and the sharp buccal hooks can be seen projecting ventrally from it. There are no conspicuous oral grooves, but radiating towards the mouth opening are some fine branched transparent lines. The antenna consists of a short wide basal segment and a small papilla apically. It is pale yellow and very lightly

chitinised. The maxillary palp is nearer the buccal hooks and consists of a short wide segment, flat at the top with a number of smaller papillae projecting from it.

There are the usual three thoracic and ten abdominal segments. The anterior border of the first thoracic segment overlaps the head and has an extra fold on the ventral side. It is covered with five to six uneven and broken rows of small brownish spines, the first row on the border being larger than the rest, especially ventrally, and projecting forwards.

The anterior spiracles occur laterally near the posterior border of the first thoracic segment. These project, one on each side, outwards and slightly upwards and end in four papillae (Fig. IX).



Figs. IV–VI. *Onesia accepta.*

Buccopharyngeal armature of larva.

Fig. IV. First stage larva. ×265.
Fig. V. Second stage larva. ×145.
Fig. VI. Third stage larva. ×80.

Lettering: *b.h.* buccal hooks, *h.p.h.* hypopharyngeal sclerite, *p.h.* pharyngeal sclerite.

The anterior border of each of the body segments is marked by a slight swelling causing an annulation and this is covered with four or five rows of very small light brown spines arranged in broken undulating lines and projecting backwards. They are equally conspicuous on the dorsal and ventral surfaces but are less developed on the sides. Ventrally on all but the first two abdominal segments there are a few rows of spines at the posterior edge of each segment as well, and the segmental line curves forwards slightly in the middle. There is no development of the swollen spiny pads which occur on the undersurface of most Calliphorid maggots.

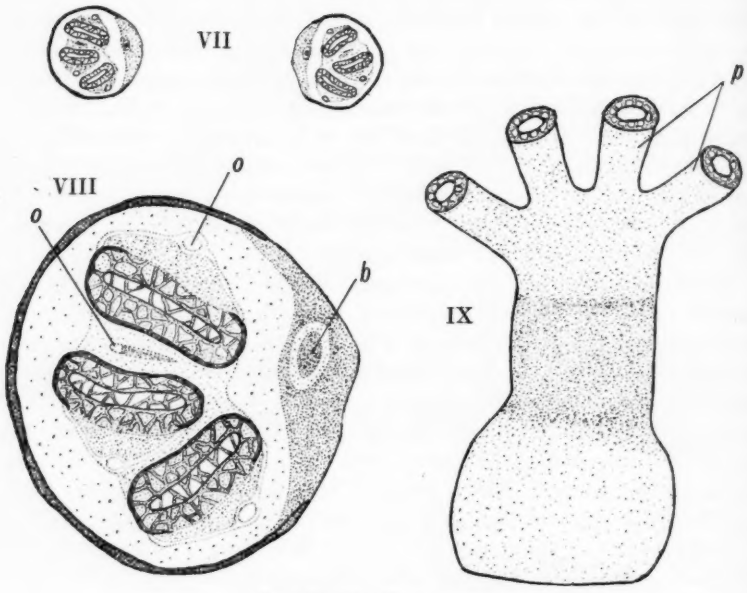
Abdominal segment 8 is not hollowed on the truncated dorsal surface but

almost flat except where the spiracular plates are situated near the anterior edge. Here there is a small depression and the felt chambers of the spiracles are visible under the thin skin. The plates are well separated and are bright yellowish. There are three pairs of papillae on the anterior edge of the truncated surface and two pairs on the lower posterior edge. There is still another pair of smaller papillae below these. Besides these usual papillae there are many small excrescences on the surface covered with tiny spines. Segment 9 is entirely ventral and consists of a small posterior portion covered with spines and a pair of large lateral projections. Between these is a pair of flaps surrounding the anus and representing the tenth segment.

Mouth-parts (Fig. VI). The pharyngeal sclerite has the same form as in the previous stage, including the pair of rods projecting forwards anteriorly over the dorsal side of the middle sclerite. The ventral surface is smooth and devoid of ridges (showing it to be of the parasitic type, see Keilin, 1915). The middle sclerite is densely chitinised and has a truncated ventral projection, as in the previous instar. The buccal hooks are comparatively much larger and stronger than in the earlier instars. The base is wide and somewhat triangular in shape. The hooks are strongly curved ventrally and sharply pointed. A narrowed curved sclerite runs between their base and the hypopharyngeal sclerite. At the ventral edge of the basal part of the hooks there are two small lightly chitinised projections. Two very small chitinous plates occur in the ventral curve of the hooks.

Posterior spiracles (Figs. VII and VIII).

The plates are separated by one and a half times the spiracular length. They are circular in outline, bulging very slightly at the button which is above the middle line instead of below it as in other Calliphorids. The peritreme is narrow and not strongly chitinised, being yellow in colour. It has no scallops or inward projections as in other blowfly maggots, being more like that of a Muscid. The peritreme does not continue to the button but gives place to thin chitin like the plate in this region. The button does not case a gap in the peritreme as in other blowfly maggots, neither is it enclosed by the peritreme as in most Muscids. It is intermediate between the two. The button itself is very pale and inconspicuous. The three slits are short and wide, nowhere reaching the peritreme. They are well separated and converge towards the button. Each slit is rounded at the ends and surrounded by a thick chitinous border which becomes thin and indistinct in the region of the perispiracular gland. The actual slit is narrow and crossed by a network of bars. The slits are fairly straight, the middle one being slightly curved upwards. The perispiracular gland openings are large, and occur in the usual positions for a *Calliphora* larva, one on the side of each slit and one between the upper and middle slits. No chitinous hairs have been detected in association with these. The general ground membrane of the plate is very thin and transparent.



Figs. VII–IX. *Onesia accepta.*

Third stage larva.

Fig. VII. Posterior spiracles. ×153.
Fig. VIII. Left posterior spiracle. ×665.
Fig. IX. Anterior spiracle. ×665. Described on p. 348.

Lettering: *b.* button, *o.* perispiracular gland opening, *p.* papillae.

The puparium (Fig. X).

This is extremely variable in size in accordance with the size of the prepupal maggot. The average length of the puparia produced in the cages was 7 mm. The puparium is reddish brown and has eleven segments. It is fairly flat on the ventral side and convex dorsally. The segments are marked off by broken or zig-zag lines and behind each are four or five rows of tiny black dots representing the bands of spines of the maggot. The segmental lines curve forwards in the centre on the ventral side. The puparium is narrow at the first segment and then widens out, being widest at abdominal segment 3 from which it again narrows down to the last segments. It is constricted at the junction of abdominal segments 1 and 2.

Segment 1 is very small and narrow and has two projections which represent the anterior spiracles of the larva. In the centre of segments 2 and 3, ventrally, the old buccopharyngeal armature of the larva can be clearly seen close against the pupal shell and lying on its side.

Ventrally the last segment shows a darker patch which represents the anus of the larva. Laterally, running the length of segments 2 and 3, are two dark ridges which represent emergence sutures. Where segments 4 and 5 meet the puparium is constricted, and there is a small concavity at each side dorsally where the chitin is thickened and darkened. At this point (Fig. X), just in front of the segmental junction, a small horn arises on each side. The tips of these are

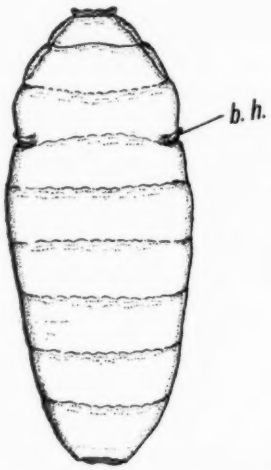


Fig. X. *Onesia accepta*. Puparium. ×9; *b.h.* breathing horn.

just visible from the ventral aspect. These curve outwards and forwards and are the nymphal breathing horns, being longer and more conspicuous than those of any other Calliphorid examined. The last segment shows two dark spots, the anterior spiracles with their three slits still discernible, and a number of dark projections representing the papillae of the eighth segment of the maggot.

VII. SUMMARY.

The life history of *Onesia accepta* Mall. is described. This species is parasitic on the earthworm *Microscolex dubius* Fletcher. The first and second larval instars are passed under the skin and the third instar in the body cavity of the host. The feeding period of the maggot is approximately 20 days, and the pupal stage about 12 days.

The external morphology of the three larval instars and of the puparium is described in detail.

ACKNOWLEDGMENT.

The writer wishes to thank Dr I. M. Mackerras for his interest and advice during the course of this study.

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EXPLANATION OF PLATE XXII.

Fig. 1. *Microscolex dubius*—normal worm.
Fig. 2. Worm parasitised by *Onesia accepta*, showing characteristic twisted appearance.
Fig. 3. Worm showing two first stage larvae under skin. (One at each side of clitellum.)
Fig. 4. Portion of worm showing second stage larva.
Fig. 5. Remains of worm with large third stage larva projecting from end.

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UNIV. OF MICH.

TWO NEW SPECIES OF TICKS FROM PANAMA (*AMBLYOMMA TAPIRELLUM* AND *A. PECARIUM*).

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(With 4 Figures in the Text.)

On March 7th, 1931, the writer collected numerous ticks (six species) from an adult tapir, *Tapirella bairdii* (Gill), soon after it had been killed near Summit, Canal Zone, Isthmus of Panama. By far the greater number belonged to a species somewhat resembling *Amblyomma cajennense* in general appearance, but showing characteristics readily separating it from that species. Two young tapirs, of the same species as the foregoing, that were examined more recently were also found to be heavily infested with this species of tick. Since this species is apparently a new one it is herewith described under the name of *Amblyomma tapirellum*.

Amblyomma tapirellum n.sp. (Figs. 1 and 2).

Male (Fig. 1). *Body:* length 2·8–4·4 mm., width 2·3–3·3 mm. Broad oval in contour; widest slightly anterior to spiracles. *Scutum:* slightly convex; anterior margins in front of eyes well rounded with the inner scapular angles long and acute; cervical grooves of medium length, deep, and slightly curved; marginal groove deep posteriorly and becoming shallower anteriorly to merge in rows of punctations behind the eyes; festoons large and well defined, longer than broad, with the interspaces wider posteriorly and tending to cause the convex area of each festoon to become slightly narrower at its posterior end. Ornate, with extensive dark brown marking on a pale background; cervical spot small and extending to the light-coloured anterior margin of the scutum; cervical stripe narrow and very irregularly outlined; ocular spot narrow; frontal spot large, irregular in outline, with small pale spots dotting its central area; falciform stripe divided in the middle; first and second lateral spots merged, the third lateral spot separated from the second; postero-median stripe broad and of medium length; postero-accessory stripes short, wide posteriorly and narrow anteriorly (somewhat triangular); second festoons practically all dark; a small light spot on median festoon; other festoons with light areas of variable size; punctations numerous, variable in size and depth, and confined mainly to the light areas, eyes rather small, flat, pale. *Venter:* brownish yellow; central area slightly rugose; many fine punctations in area

posterior to anal region; genital orifice opposite posterior margin of coxa II; festoons with strongly chitinised scutes extending beyond the posterior margin of body as prominent thin-edged plates of sub-equal size; some specimens bear small and rather ill-defined brownish plaques bordering on the anterior margins of the median, second and fourth scutes; median plaque elongate

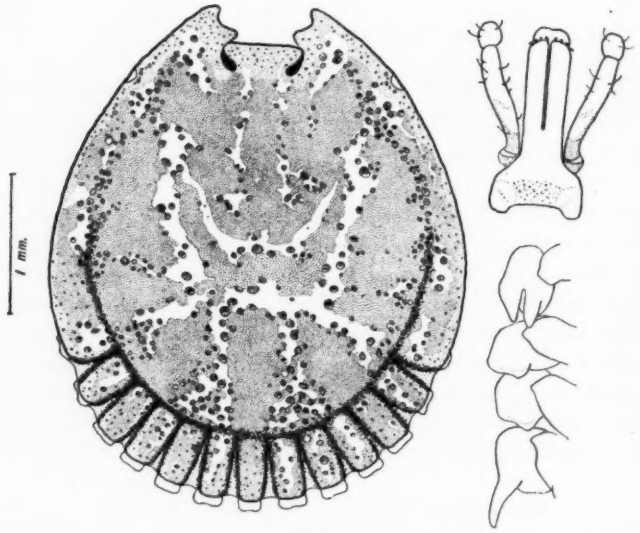

Fig. 1. *Amblyomma tapirellum* n.sp. ♂. Scutum, capitulum in dorsal aspect, coxae I–IV (K.R. del.).

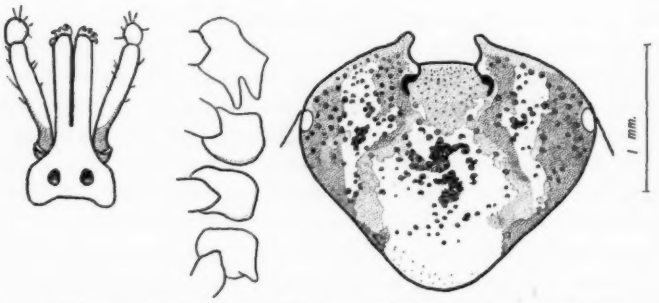

Fig. 2. *Amblyomma tapirellum* n.sp. ♀. Scutum, capitulum in dorsal aspect, coxae I–IV (K.R. del.).

and extends to more than half the distance from the scute to the anal groove; plaques in front of second scutes are rather ovoid in shape while those bordering on the fourth scutes are smaller and more circular. Spiracles comma-shaped and of medium size *Capitulum:* length 1·1–1·3 mm. Basis sub-rectangular,

about twice as broad as long, with posterior angles moderately salient and rounded, somewhat indistinct pale markings along posterior margin of basis; palps of medium length, slender, with article 2 about twice as long as article 3 and with pale ornamentation on article 2. Hypostome dentition 3 | 3. *Legs:* of medium length, rather stout, brown, with slight pale enamelling on dorsal surface of femora and tibiae; coxa I with two stout spurs of moderate length, the external spur slightly the longer; coxae II and III each with a single, short, broad, plate-like spur; coxa IV bears a single, long, pointed spur.

Female (Fig. 2). *Body:* length 3·2–4·8 mm., width 2·6–3·7 mm. Broad oval in contour, widest at middle. Dorsum pale brown with dark reddish brown markings; marginal groove continuous and rather shallow; festoons well defined, the fourth one on either side slightly shorter anteriorly than the others; many coarse punctations present. *Scutum:* somewhat broader than long and ranging from $2 \times 2{\cdot}6$ mm. to $2{\cdot}1 \times 2{\cdot}8$ mm.; triangular, with the anterior margins rounded; scapular angles long and acute; postero-lateral margins slightly convex with the posterior angle broad and well rounded; cervical grooves short, deep, slightly curved and becoming more shallow and divergent posteriorly. Ornate, with dark brown markings on a pale yellow ground; anterior margin pale; a dark band extends between the ocular spot and the limiting spot on each postero-lateral margin; limiting spots somewhat large and causing a slight, but noticeable, and constant indentation in each lateral border of the pale median marking a short distance from the posterior border; ocular spots large; cervical stripes extend posteriorly to merge with the limiting spots; cervical spots large; punctations numerous, of varying size, becoming finer near posterior angle; eyes large, flat, pale. *Venter:* light brown; punctations few and small; genital aperture opposite the interspaces between coxae II and III; dividing grooves between festoons shallow but well defined. Spiracles of medium size and comma-shaped. *Capitulum:* length 1·2–1·6 mm.; basis form as in ♂; porose areas of medium size, sub-oval, well separated, slightly divergent; palps of medium length, slender with article 2 about twice as long as article 3, ornate as in ♂. Dentition 3 | 3. *Legs:* of medium length, somewhat stout, brown; coxa I with two stout spurs, the external one the longer and more slender; coxae II and III each with a short, broad, plate-like spur; coxa IV with a short triangular spur.

Type Host: Tapir, *Tapirella bairdii* (Gill).

Type Locality: Summit, Canal Zone, Isthmus of Panama.

The *holotype* (♂) and *allotype* (♀) will be deposited in the United States National Museum, Washington, D.C. *Paratypes* are deposited in the collection of the Gorgas Memorial Laboratory, Panama, R. de P., and in the author's collection.

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On April 11th, 1932, a native hunter killed a collared peccary, *Pecari angulatus bangsi* Goldman, in the Miraflores area of the Canal Zone, Isthmus of Panama. Ticks (24 ♂♂, 1 ♀) that were taken from this peccary and sent to

this laboratory appear to be a previously unknown species which is now described under the name of *Amblyomma pecarium* as follows:

Amblyomma pecarium n.sp. (Figs. 3 and 4).

Male (Fig. 3). *Body:* length 2·9 mm., width 2·2 mm. Broad oval in contour, somewhat narrower anteriorly and becoming broadest posterior to the middle. *Scutum* with a markedly depressed area on either side beginning a short distance behind the eyes as narrow depression and extending posteriorly and very gradually deepening and broadening to about the second festoon where it ends rather abruptly; the area of more normal elevation between these depressions narrows posteriorly in the postero-median field and rises to form an elongate, high, narrow, keel-like ridge that extends back to sink and merge in the marginal groove; the cervical grooves short, broad, of medium depth;

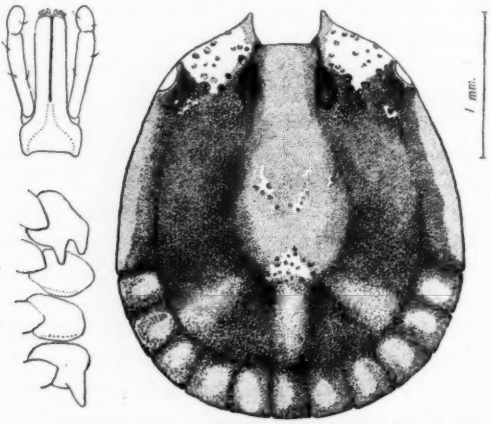

Fig. 3. *Amblyomma pecarium* n.sp. ♂. Scutum, capitulum in dorsal aspect, coxae I–IV (K.R. del.).

marginal groove deep and well defined at posterior half of scutum, but nearly obliterated anteriorly by the depressed areas; festoons slightly longer than broad, well defined, with separating intervals of medium width; a few very small, shallow punctations are present on the anterior scapular angles. Nearly inornate; brown, with a dull gloss and considerable transparency; median field lighter brown, darker markings along margins and on festoons; ornamentation confined to a light spot in each scapular field and a spot in the median field at anterior end of the keel-like ridge as shown in Fig. 3. Eyes pale and flat. *Venter:* pale yellow; with some very fine punctations; genital aperture opposite posterior margin of coxa II. Spiracles comma-shaped, of medium size. Festoons well defined and the postero-ventral margin of each terminating in a prominent triangular-shaped scute. *Capitulum:* length 1·2 mm. Basis sub-rectangular with the postero-lateral angles very slightly salient; palps long and slender, with article 2 nearly thrice as long as article 3. Hypostome

dentition 3 | 3. *Legs:* brown, with considerable transparency; coxa I with two well separated spurs of medium length, the outer spur but slightly longer and more slender than the inner; a broad, curved ridge on each of coxae II and III; a moderately long, pointed spur on coxa IV.

Female (Fig. 4). *Body:* length 3·8 mm., width 3·3 mm. Contour subcircular, being nearly as broad as long with the posterior border broadly rounded. Reddish brown. Dorsum with numerous coarse punctations with fine creases extending between many of them producing a pitted and wrinkled appearance; marginal groove shallow and tending to become indistinct posterior to the first festoon; festoons fairly well defined with the anterior border of each irregular in outline. *Scutum:* relatively large, 2·1 × 2·3 mm., triangular, with antero-lateral margins rounded and scapular angles markedly pointed; posterior angle truncate and the postero-lateral margins rectilinear; cervical grooves short, deep anteriorly and extending posteriorly as shallow depressions that diverge outwards and end beyond the middle of the scutum; the whole

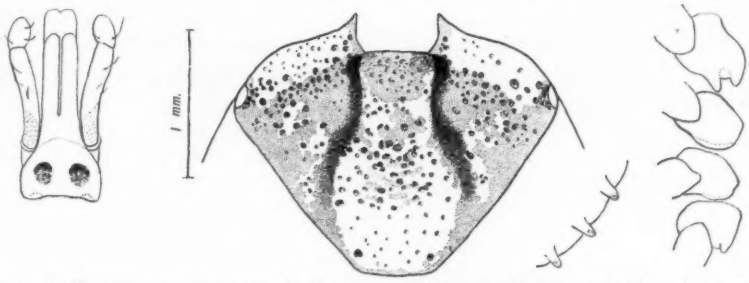

Fig. 4. *Amblyomma pecarium* n.sp. ♀. Scutum, capitulum in dorsal aspect, three festoons in ventral aspect to show tubercles, coxae I–IV (K.R. del.).

median field between the cervical grooves is elevated above the surface of the lateral fields as a convex ridge. Ornate, with a broad, elongate, pale yellow spot covering the median field except where it approaches the emargination; at each antero-lateral field a prominent pale yellow spot extends from the eye inwards to include the scapular angle; a broken, irregular, yellow stripe extends down each lateral field to end at the postero-lateral margin beyond the middle of the scutum; the ground colour not covered by the yellow markings is almost a seal brown and transparent; an elongate group of small punctations extends across each scapular angle behind the eye and the light antero-lateral spots; numerous fine scattered punctations occur on the median field; eyes moderately large, pale, nearly flat. *Venter:* light yellowish brown; many fairly large punctations on the festoons and in the post-anal area; genital aperture opposite the interspace between coxae II and III; festoons somewhat rugose and ill defined; eight of the festoons each bears a rather long and somewhat conspicuous chitinous tubercle at its postero-internal angle; these tubercles on either side are directed inward. Spiracles comparatively narrow and elongate comma-shaped. *Capitulum:* length 1·6 mm.

Basis sub-rectangular, lateral margins slightly convex and the postero-lateral angles not definitely salient; porose areas large, oval, nearly parallel, with the interval between narrower than the diameter of one of the areas; palps long, slender, and somewhat flattened laterally; article 2 about two and a half times as long as article 3. Hypostome long and narrow, dentition 3 | 3. *Legs:* reddish-brown with slight transparency; moderately long and somewhat stout; coxa I with two well separated, unequal spurs, the external spur being of medium length, slender, and pointed, the internal spur being short and blunt; coxae II and III each with a low, broad, curved ridge; coxa IV bears a short, blunt spur, about one and a half times as long as broad.

Type Host: Collared peccary, *Pecari angulatus bangsi* Goldman.

Type Locality: Miraflores, Canal Zone, Isthmus of Panama.

The *holotype* (♂) and *allotype* (♀) will be deposited in the United States National Museum, Washington, D.C. *Paratypes* are deposited in the collection of the Gorgas Memorial Laboratory, Panama, R. de P., and the author's collection.

The writer is greatly indebted to Dr L. E. Robinson for his kindness in examining specimens of the two species of ticks described in this paper and for his opinion regarding their validity as new species.

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STUDIES ON SOME NEW OPHIDIAN AND AVIAN COCCIDIA FROM UGANDA, WITH A REVISION OF THE CLASSIFICATION OF THE EIMERIIDEA.

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(With 6 Figures in the Text.)

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I. Introduction.

During my stay in Uganda, between 1927 and 1929, while working at the Human Trypanosomiasis Institute, Entebbe, I examined about four hundred vertebrate animals for parasitic Protozoa[1]. Out of this number eleven individuals were found to harbour Coccidia.

In the present paper an account is given of the Coccidia occurring in three species of snakes, *Psammophis sibilans*, *Bitis arietans*, *Boaedon lineatus*, and in a bird, *Phalacrocorax carbo lugubris*[2]. Coccidia have not been previously recorded from any of these hosts. The ones described in this paper are all new to science: for one of them it was necessary to create a new genus (*Wenyonella*), while another belongs to a genus hitherto represented by two species (*Caryospora*), the two others being new species of well-known genera (*Isospora*, *Eimeria*).

Another coccidium was recovered from a bat, *Chaerephon* (= *Nyctinomus*) *limbatus*. It proved to be identical with *Eimeria dukei* Lavier 1927, obtained from *C.* (= *N.*) *pumilus*, in the same locality (Entebbe).

[1] The blood parasites were dealt with in a previous publication (Hoare, 1932).

[2] For the identification of the snakes and the bird I am indebted to Mr H. W. Parker, of the British Museum (Natural History Department), and to Captain C. R. S. Pitman, D.S.O., M.C., Game Warden of the Uganda Protectorate, respectively.

The infected animals were in every case brought to the laboratory alive. Some were kept under observation for a few days, while others were sacrificed and dissected shortly after their capture. The exogenous stages of development of the Coccidia—which have to be observed on fresh material—were dealt with on the spot, while the endogenous stages were studied from the fixed organs on my return to England.

Methods employed. The droppings or intestinal contents of the animals in which oocysts or sporocysts of Coccidia had been detected were mixed with 1 per cent. solution of chromic acid and kept in Petri dishes. The sporogony was then followed from day to day until the final stage of development was reached. The organs that served for the study of the endogenous stages were fixed in Dobell's alcoholic modification of Bouin's picroformol (sat. sol. of picric acid in 90 per cent. alcohol—75 parts; 40 per cent. formaldehyde—25 parts; glacial acetic acid—5 parts) for about 18 hours, and were then transferred to 70 per cent. alcohol in which—after several changes—they were preserved till required for sections. The intestine—which proved to be the only organ infected—was in most cases fixed *in toto*, and portions of it, cut out at different levels, were used for sections. The method of embedding (suitable for any tissue fixed in Bouin's alcoholic solution and measuring about 5 c.mm.) was as follows: In 90 per cent. alcohol—overnight; in absolute alcohol—6 hours (changed once); in mixture of equal parts of chloroform and carbon bisulphide—overnight (changed once); in saturated solution of paraffin wax in carbon bisulphide—6 hours (kept warm on the top of the paraffin oven); in pure paraffin wax—overnight.

Before staining, the sections were kept in 70 per cent. alcohol for at least 24 hours, in order to extract from them all traces of picric acid. They were then stained with Mayer's acid haemalum and counterstained with eosin.

II. Description.

(1) **Caryospora legeri** sp.n.

Fig. I.

A coccidium belonging to the genus *Caryospora* was found in the "Hissing Sand Snake," *Psammophis sibilans*, which is very rare in the neighbourhood of Entebbe. Only three specimens were examined, and two of them proved to be infected.

In both cases oocysts with unsegmented zygotes were recovered directly from the voided faeces and from the rectal contents.

The further development (sporogony) of the parasite was observed in the chromic acid solution.

The oocysts are subspherical or slightly ovoid, with a thick, greyish coloured wall, consisting of two layers. At one of the poles of the oocyst a minute funnel-shaped micropyle can sometimes be made out. The measurements of the oocysts vary between $20{\cdot}8 \times 19{\cdot}2\,\mu$ and $30{\cdot}4 \times 25{\cdot}6\,\mu$. The zygote is usually spherical, with a diameter from $12{\cdot}8$ to $14{\cdot}4\,\mu$, and contains a number of large refringent globules (Fig. I, 10). Since the zygote gives rise to a single sporocyst with its eight sporozoites, it constitutes the only sporoblast.

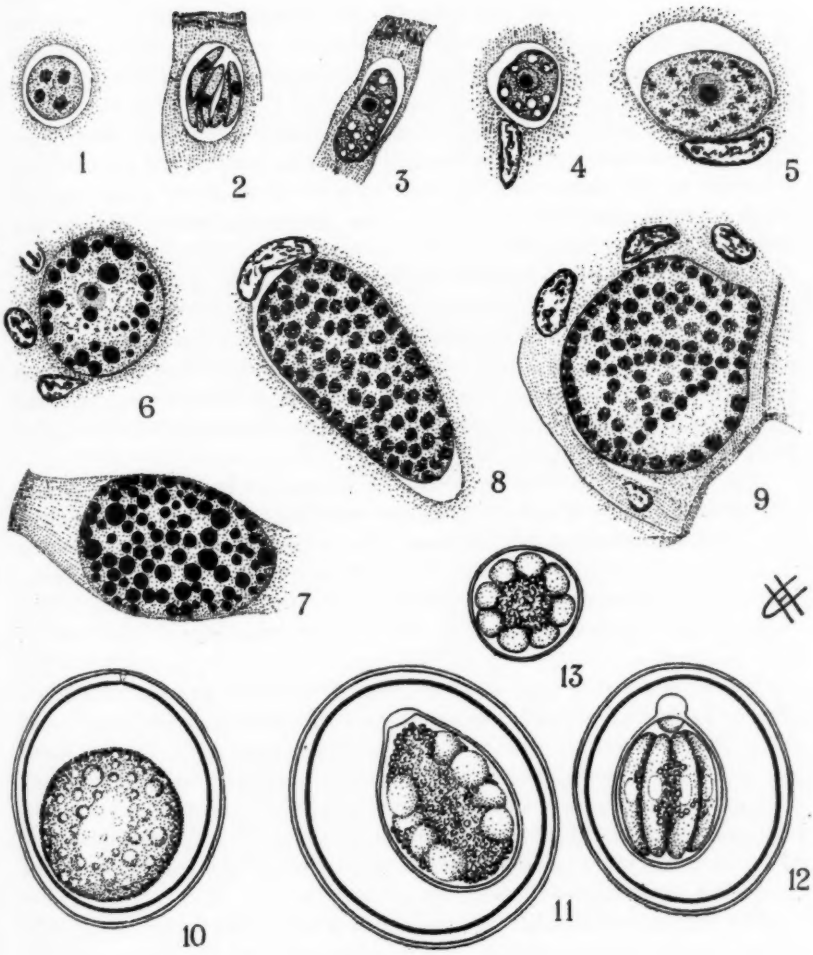

Fig. I. *Caryospora legeri* sp.n.

1–9. Endogenous stages (sections of small intestine) (×1500): 1, schizont; 2, merozoites; 3, 4, young macrogametocytes; 5, growing macrogametocyte; 6, 7, mature macrogametocytes (viewed from surface in 7); 8, 9, microgametocytes.—10–13. Exogenous stages (sporogony) (living specimens) (*ca.* ×1800): 10, oocyst (showing micropyle) with unsegmented zygote; 11, oocyst with budding sporoblast invested with sporocyst-wall; 12, oocyst with mature sporocyst; 13, optical transverse section of sporocyst, showing arrangement of eight sporozoites around residual body. (All the figures were drawn with the aid of a camera lucida.)

On the second day after the oocysts were isolated the sporocyst is formed. At this stage of development it is piriform and has a lensiform thickening at the narrower end. By this time the sporoblast (= zygote), which consists of a finely granular mass, filling almost the entire sporocyst, has produced eight rounded, homogeneous buds, the precursors of the sporozoites (Fig. I, 11).

On the third day the sporocyst reaches its final development. Its piriform shape of the preceding day has changed to regular ovoid, while the lensiform structure at the narrow pole has become a pronounced globe-like "knob." On the inner surface of the sporocyst, directly under the "knob," there is now a hemispherical "plug" (Fig. I, 12).

The mature sporocysts measure from $16{\cdot}4 \times 11{\cdot}2\,\mu$ to $19{\cdot}2 \times 13{\cdot}6\,\mu$. Each of them contains eight spindle-shaped sporozoites, measuring about $11\,\mu$ in length. These are arranged longitudinally in a regular manner, like staves of a barrel ("en barillet"), around a bulky granular residual body, part of which protrudes between the sporozoites (Fig. I, 12). The number of sporozoites and their arrangement in relation to the sporocystic residue are best seen in optical transverse sections (Fig. I, 13).

The endogenous cycle of development takes place in the small intestine. The entire intestine of one of the snakes was fixed, and portions, cut out at different levels, were used for sections. The stages of development were extremely scanty, but a sufficient number were observed to form a general idea of their appearance and localisation. Both schizogony and the initial stages of sporogony (gametogony) take place within the epithelial cells of the villi.

The schizonts seen were small rounded bodies with a diameter of $7{\cdot}6\,\mu$ (Fig. I, 1). The number of merozoites produced probably does not exceed eight. They are crescentic, measuring about $7{\cdot}6\,\mu$ in length. The nucleus, which appears to be of the massive type, is situated near the middle of the body (Fig. I, 2). Since the majority of stages seen in this snake were gametocytes, it is possible that the type of schizogony observed appears only towards the end of the asexual cycle and that schizonts of a different type may occur during the earlier stages of the infection.

The young macrogametocytes are almost equal in size to or only slightly larger than the mature schizonts. Their bodies are either irregularly rounded, measuring about $7{\cdot}6 \times 5{\cdot}7\mu$, or elongated, measuring about $9{\cdot}5 \times 4{\cdot}7\mu$ (Fig. I, 3, 4). The nucleus of these forms is of the vesicular type, with a large, slightly excentric karyosome and no visible signs of chromatin in the space between the karyosome and the nuclear membrane. The cytoplasm of the young female gametocytes stains deeply and contains a number of vacuole-like inclusions. Later the macrogametocyte increases in size, to about $13{\cdot}3 \times 9{\cdot}5\,\mu$, its cytoplasm stains lightly, but in place of the clear vacuoles there appear irregular dark inclusions (Fig. I, 5). When the macrogametocyte (= macrogamete?) reaches maturity it is spherical or ovoid, the former type having a diameter of

about 13·3 μ, and the latter measuring about 19·0 × 13·3 μ (Fig. I, 6, 7). In these forms the cytoplasm—mainly on the periphery—contains numerous refractile globules, staining darkly, almost black. The staining properties and the cytoplasmic inclusions of the forms described, together with the nuclear structure, are very characteristic of developing coccidian macrogametocytes. The changes taking place in the cytoplasm can be interpreted as follows: in the early stages the reserve material (volutin?) is in a diffuse state in the vacuolated cytoplasm, causing the latter to stain deeply; later the reserve material becomes concentrated in the form of irregular dark granular masses—probably within the vacuoles, which are no longer in evidence—throughout the cytoplasm which now ceases to stain deeply; and finally the inclusions assume the characteristic globular shape described above and accumulate chiefly on the surface of the parasite (Fig. I, 3–7).

The microgametocytes are either elongated bodies, measuring about 26·6 × 11·4 μ, or irregularly rounded, 22·8 × 20·9 μ, with numerous compact nuclei arranged densely on the surface of the parasite (Fig. I, 8, 9). The production of microgametes has not been observed.

In comparing the schizonts with the gametocytes, the most notable feature is the striking difference in size between these stages, the schizonts being the smallest and the microgametocytes the largest. A similar difference in the dimensions of these stages has, however, been frequently observed in other Coccidia.

For nearly thirty years the only known representative of the genus *Caryospora* was *C. simplex* Léger 1904, parasitic in the intestine of the viper, *Vipera aspis*, in France. A full description of this parasite appeared some years after its discovery (Léger, 1911). A second species of this genus, *Caryospora brasiliensis*, from a Brazilian snake, *Chlorosoma aestivum*, was recently reported by Carini (1932).

The genus was originally named *Karyospora* (Léger, 1904), but in the second paper (Léger, 1911) the author referred to it as *Caryospora*, without giving any reasons for the change in spelling. Since this transliteration is in accordance with the Rules of Zoological Nomenclature and the name, as it now stands, is more familiar, it is best not to apply the Law of Priority in this case. It is realised, however, that this point is debatable. Thus, in the case of *Karyolysus* Labbé, 1894, the name was emended to *Caryolysus* by the author himself (Labbé, 1899), but subsequent writers reverted to the original spelling, which is the one generally accepted at present.

The form described in this paper differs considerably from the type species and from *Caryospora brasiliensis*, as can be seen from Table I, in which the most important differential characters of the three species of *Caryospora* are given.

I propose to name the coccidium of the African snake *Caryospora legeri* sp.n. in honour of Prof. Louis Léger, who created the genus of which the new species is the third representative.

Table I. *Diagnostic characters of the species of* Caryospora.

Species of *Caryospora* and their hosts	Exogenous stages: Dimensions in microns: Oocyst	Exogenous stages: Dimensions in microns: Sporocyst	Exogenous stages: Sporocystic residue	Exogenous stages: Arrangement of sporozoites	Endogenous stages (dimensions in microns): Schizont	Endogenous stages (dimensions in microns): ♀ gametocyte	Endogenous stages (dimensions in microns): ♂ gametocyte
C. simplex Léger 1904 (host: *Vipera aspis*)	10·0 × 10·0–15·0 × 15·0	12·0 × 8·0	Small	Regular ("en barillet") or spiral	8·0 × 8·0–10·0 × 10·0	12·0 × 12·0–15·0 × 15·0	6·0 × 6·0–9·0 × 9·0
C. brasiliensis Carini 1932 (host: *Chlorosoma aestivum*)	20·0 × 20·0–22·0 × 22·0	17·0 × 13·0	Small	Irregular	?	?	20·0 × 20·0
C. legeri sp.n. (host: *Psammophis sibilans*)	20·8 × 19·2–30·4 × 25·6	16·4 × 11·2–19·2 × 13·6	Large	Regular ("en barillet")	7·6 × 7·6	13·3 × 13·3–19·0 × 13·3	26·6 × 11·4–22·8 × 20·9

As is seen from Table I, the oocysts of *C. legeri* are practically double the size of those of *C. simplex*, and somewhat larger than those of *C. brasiliensis*. There is also a corresponding difference in the dimensions of the sporocysts of the three species. They also differ in the size of the sporocystic residue, which is quite insignificant in *C. simplex* and *C. brasiliensis*, but bulky in the new species. The arrangement of the sporozoites within the sporocysts is also of diagnostic value: it is regular ("en barillet") in *C. simplex* and *C. legeri*, and quite irregular in *C. brasiliensis*.

There are other minor points in which the three forms differ (some of which will be found in Table I), but those indicated above are sufficient to characterise the new species. It may be noted here that, according to Léger, it took the zygotes of *C. simplex* about a month to sporulate. This is, doubtless, due to the fact that he kept the infected faeces in a moist chamber without the addition of any antiseptic fluid. Under such conditions the sporogony of Coccidia generally proceeds very slowly, many of the oocysts showing various abnormalities or perishing before they reach maturity.

Diagnosis.

Systematic position. Caryospora legeri sp.n. (Coccidiida Eimeriidae).

Description. Oocysts subspherical or ovoid, with micropyle: 20·8 × 19·2–30·4 × 25·6 μ; sporocysts ovoid, with "knob" and "plug" at narrow pole: 16·4 × 11·2–19·2 × 13·6 μ; sporozoites arranged regularly around large sporocystic residue: sporulation time, 2 days; unsegmented oocysts discharged from host.

Habitat. Small intestine (intra-epithelial) of *Psammophis sibilans* (Ophidia Colubridae).

Locality. Entebbe, Uganda.

(2) **Isospora dirumpens** sp.n.

Fig. II.

The examination of fifteen specimens of the "Puff-Adder," *Bitis arietans*, captured at various times near Entebbe, revealed a coccidial infection in four individuals. The parasites found in the faeces appeared in the form of cysts, each containing four vermicules and a residual body (Fig. II, 8, 9, 11, 12) The cysts were suggestive either of the oocysts of a *Cryptosporidium* or of the

isolated sporocysts of an *Isospora*. The finding of a number of immature oocysts in one snake confirmed the latter diagnosis.

The sporocysts of the *Isospora* were recovered both from the natural droppings of the snakes kept in captivity and from the rectal contents on dissection, so that it is evident that the parasite leaves its host in the form of fully developed isolated sporocysts.

The sporocysts have a fairly regular ellipsoid shape, and a thick, somewhat yellowish wall without any special structures at the pole. They measure from $10{\cdot}4 \times 8{\cdot}0$ to $11{\cdot}2 \times 9{\cdot}6\,\mu$. The four sporozoites are sausage-shaped, with one end somewhat narrower than the other, and measure just over $7\,\mu$ in length. They are arranged without any order in a group lying to one side of a massive residual body. The latter is composed of coarse refringent globules either agglomerated into one rounded mass or spread out irregularly (Fig. II, 8, 9, 11, 12).

As stated above, unruptured oocysts were seen only in one snake. The majority of them contained mature sporocysts, but a few were in the earlier stages, from which it is possible to reconstruct their development.

The oocysts with unsegmented zygotes—which were extremely scanty—have an ellipsoid shape and measure about $15{\cdot}2 \times 11{\cdot}2\,\mu$. The zygote which fills almost the entire cyst has a coarsely granular appearance (Fig. II, 3). The two sporoblasts, when first formed, are large ovoid bodies with a granular cytoplasm (Fig. II, 4), which later become invested by a very thin membrane (sporocyst) (Fig. II, 5). There is no oocystic residue. The further development of the sporoblasts is accompanied by an increase in size and a considerable thickening of the sporocyst wall. The sporozoites are rounded in the early stages (Fig. II, 6), but later become sausage-shaped, and are grouped to one side of the bulky residual body.

One of the most striking peculiarities of this coccidium is its oocyst. Its wall is extremely delicate and elastic, being more like a soft membrane than a rigid capsule. On account of this, no definite shape can be ascribed to the oocyst, since it moulds itself first around the zygote and later around the sporocysts. By degrees, as the latter develop, the membrane is stretched out, and at the stage when the contents of the sporocysts are almost ripe, nothing is visible of the oocyst but two fine incurved lines connecting the two sporocysts (Fig. II, 6, 7). Owing to the flexibility of the oocyst its dimensions are variable: at the stage just described it measures from $16{\cdot}0$ to $16{\cdot}8\,\mu$ in length and about $9{\cdot}6\,\mu$ in breadth. The free sporocysts are usually markedly larger than those enclosed by the oocyst, each being but slightly smaller than the zygote from which the two originated. Hence it would appear that the rupture of the membrane is to a certain extent due to its being overstrained by pressure of the growing sporocysts. A number of ruptured oocysts—empty or containing a single sporocyst—were seen in the faeces of one of the snakes (Fig. II, 10).

The endogenous cycle of development of this parasite takes place in the small intestine of the snake. Almost the entire intestines of the four infected snakes were fixed and sections were made from various portions. Practically

the only stages of the parasite present were fully developed oocysts, containing two sporocysts with four sporozoites in each. Only isolated specimens of unsegmented zygotes were observed. In the hope of securing all the stages of development artificial infection of one young puff-adder was attempted. Unfortunately, the snake that served for the experiment was already naturally infected with the coccidium in question, as manifested by the presence of sporocysts in its droppings. This snake was fed four times in the course of one week on washed ripe sporocysts from another snake, after which it was killed and its entire intestine fixed. On examination of sections nothing but fully developed oocysts could be found, as in the case of the other naturally infected snakes. This would indicate that the snake had probably acquired an immunity to re-infection.

In one of the infected snakes no Coccidia could be found in sections of the intestine, whereas in the other three it was heavily infested. The parasites occurred both inside the epithelial cells and in the subepithelial tissues (Fig. II, 1, 2). In the latter case they were sometimes densely packed in a continuous layer immediately beneath the epithelium, extending into the lamina propria and the lymph nodules (solitary follicles) of the villi. They were not seen, however, in the submucosa. It may be noted here that, although the parasites occurred both inside and under the epithelium in all the three snakes, in one of these the majority were intra-epithelial (Fig. II, 2), in another they were more numerous in the subepithelial tissues (Fig. II, 1) while in the third their distribution in both situations was more or less uniform.

Since the sporogony is completed in the host tissues and only free sporocysts can usually be recovered from the faeces, it would appear that the oocysts generally rupture *in situ*, and the sporocysts alone are liberated into the lumen of the intestine, probably as the result of portions of the epithelium being shed off. This, probably, always takes place when the oocysts develop beneath the epithelium. In the case of those developing within the epithelium it is conceivable that sometimes the cast-off host cells harbouring the oocysts are digested and the oocyst remains intact, its rupture taking place in the lumen of the intestine. This would explain the rare occurrence of the unruptured oocysts referred to above. The rupture of the thin membrane might be facilitated by friction against the lime particles and hairs present in the excrement of the snakes.

A similar type of development, when the parasite completes its life cycle before it leaves the tissues of its host, has been described for some other species

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Legend to Fig. II.

Isospora dirumpens sp.n.

1, 2, sections of villi of small intestine showing mature oocysts in subepithelial tissue (1), and within epithelium (2); 3, oocyst with unsegmented zygote; 4, oocyst with two naked sporoblasts; 5, oocyst with sporoblasts invested with thin sporocyst; 6, 7, sporocysts (containing immature sporozoites) within outstretched oocyst-membrane; 10, ruptured oocyst with one sporocyst; 8, 9, 11, 12, free mature sporocysts (as they appear in faeces). (Figs. 3–12 are from living specimens. All the figures were drawn with the aid of a camera lucida. Magnifications: 1, 2, ×750; 3–12, ×2500.)

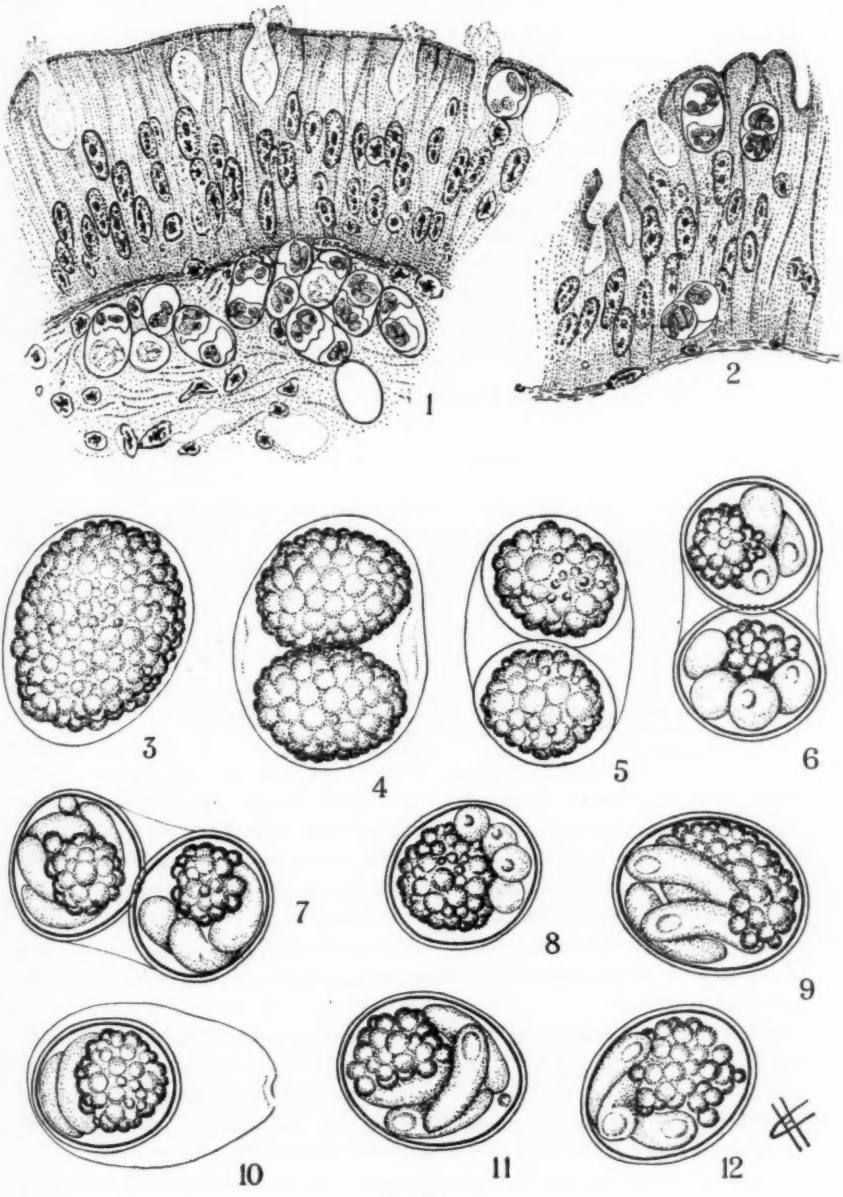

Fig. II.

of *Isospora*. Thus, it occurs in *I. bigemina* of cats and dogs (Wenyon, 1923; Wenyon and Sheather, 1925), in *I. laverani* of a snake (Hagenmuller, 1898), and in *I. buteonis* of hawks and owls (Henry, 1932). In all these cases the oocyst wall is extremely thin and fragile, as in the parasite of the puff-adder, and the oocyst ruptures while still in the intestine of the host, liberating the sporocysts into the lumen.

The facts recorded seem to indicate that the type of oocyst produced by Coccidia depends on the site where its development takes place. It is thin-walled when the sporogony is completed within the tissues of the host, and thick-walled when the final stages of sporogony occur either in the lumen of the intestine or outside the body of the host. A striking example of this adaptation is recorded for *I. bigemina* by Wenyon and Sheather (1925) and Wenyon (1926). In acute infections of the dog, the reproduction of this coccidium takes place within the epithelium of the intestine, and immature

Table II. *List of species of* Isospora *recorded from snakes and lizards.*

Host	*Isospora* spp.	Author	Dimensions in microns: Oocysts	Dimensions in microns: Sporocysts
OPHIDIA:				
Bitis arietans	*I. dirumpens*	Hoare, sp.n.	15·2 × 8·0–16·8 × 9·6	10·4 × 8·0–11·2 × 9·6
Coronella austriaca	*Isospora* sp.	Grassi, 1881	?	7·0 × 4·5
Coelopeltis lacertina	*I. laverani*	Hagenmuller, 1898	?	12·0 × 10·0
Vipera aspis	*I. fragilis*	Léger, 1904	?	?
Crotalus confluentus	? *I. crotali*	(Triffitt, 1925) em.	?	10·0 × 10·8–12·5 × 11·0
Naja flava	*I. naiae*	Fantham, 1932	13·6 × 8·2–20·0 × 14·3	8·6 × 5·6–13·3 × 10·0
LACERTILIA:				
Gongylus ocellatus	*I. camillerii*	Hagenmuller, 1898	22·0 × 22·0	18·0 × 12·0
Chamaeleo vulgaris	*I. mesnili*	Sergent, 1902	30·0 × 30·0	16·0 × 10·0
Agama colonorum	*Isospora* sp.	Wenyon, 1926	?	?
Varanus griseus	*Isospora* sp.	Adler (*fide* Wenyon, 1926)	?	?

forms with *thick*-walled oocysts are passed in the faeces. In chronic infections this parasite reaches maturity in the subepithelial tissues and escapes into the lumen of the intestine, and thence into the outer world, in the form of fully developed *thin*-walled oocysts. Frequently the oocyst ruptures and the sporocysts alone are found in the faeces. The *Isospora* of the puff-adder differs from that of the dog in that it invariably completes its sporogony within the tissues of the host and produces thin-walled oocysts both in the intra- and subepithelial positions.

The number of Coccidia of the genus *Isospora* recorded from reptiles is very small, as is seen from the list given in Table II, which, as far as could be ascertained, is complete up to 1932[1], and includes the species described in this paper.

Among the species named in the foregoing list only five or six, not to mention the one from the puff-adder, are sufficiently characterised to be accepted as valid: they are *Isospora* sp. from *Coronella*, *I. laverani*, *I. camillerii*, *I. mesnili*, *I. naiae* and possibly *I. crotali*. What were probably the sporocysts

[1] Lists of the species of *Eimeria* recorded from reptiles were published by Triffitt (1925) and by Wenyon (1926).

of an *Isospora* were described by Triffitt (1925) as oocysts of a *Cryptosporidium* (*C. crotali*), but as the identity of this parasite cannot be definitely established without a knowledge of the earlier stages of its sporogony, its classification in the genus *Isospora*, first suggested by Wenyon (1926), should be regarded as provisional. Apart from the species noted in Table II, a coccidium found by Galli-Valerio (1926) in a snake, *Coelopeltis lacertina*, was also included in the genus *Isospora*, under the name *I. coelopeltis*. Since the characters attributed to this parasite (four sporocysts with two sporozoites in each) are those of the genus *Eimeria*, it is obvious that a *lapsus calami* had occurred. Prof. B. Galli-Valerio, whom I approached on this subject, kindly informed me that the parasite was actually an *Eimeria* and that the name was corrected accordingly in the reprints of his paper. The coccidium, therefore, becomes *Eimeria coelopeltis* (Galli-Valerio, 1926).

The *Isospora* described in this paper cannot be identified with any of the reptilian Coccidia listed in Table II, though the dimensions of its sporocysts approach those of *I. laverani*, *I. naiae* and *I. crotali*. The last named, the ambiguous nature of which has already been pointed out, differs from the present form in the smallness of its sporocystic residue. As regards the first, it has many points in common with the new parasite. Thus, *I. laverani* completes its development in the subepithelial tissues of the intestine; its oocyst is represented by a delicate, fragile membrane which usually ruptures early and liberates the sporocysts. These, however, differ from the sporocysts of the *Isospora* of the puff-adder in their subspherical shape, and the *regular* arrangement of the sporozoites around a *small* residual body. The difference in the dimensions of the sporocysts of these parasites is, however, insignificant. *I. naiae* differs from the new form mainly in the presence of an oocystic residue and in the absence of a sporocystic one.

The coccidium from the puff-adder is sufficiently well characterised to be regarded as a distinct species, which I propose to name *Isospora dirumpens* sp.n.

Diagnosis.

Systematic position. *Isospora dirumpens* sp.n. (Coccidiida Eimeriidae).

Description. Oocysts thin and flexible (shape inconstant): 15·2 × 11·2–16·8 × 9·6 μ. Sporocysts ellipsoid, without polar differentiation: 10·4 × 8·0–11·2 × 9·6 μ; sporozoites arranged irregularly; large sporocystic residue; mature, free sporocysts discharged from host.

Habitat. Small intestine (intra- and subepithelial) of *Bitis arietans* (Ophidia Viperidae).

Locality. Entebbe, Uganda.

(3) **Wenyonella africana** gen.n., sp.n.

Fig. III.

A coccidial infection was discovered in two out of twelve specimens of the "Brown House Snake," *Boaedon lineatus*, examined. The snakes were captured in Entebbe, where they are very common.

Scanty oocysts with unsegmented zygotes were recovered from the contents of the cloaca and rectum of these snakes after dissection. The oocysts are ovoid or subspherical in shape, measuring from 18·5 × 16·0 to 19·2 × 17·6 μ. The wall is thick and consists of two layers, the inner of which has a yellowish tinge, while the outer one is colourless. I was unable to detect a micropyle (Fig. III, 7).

The zygote is more or less spherical, with a diameter from 13·6 to 14·4 μ, and is filled with numerous small granules. After remaining in chromic acid one day the zygote gives rise to four sporoblasts, leaving no oocystic residue (Fig. III, 8). After two days the sporoblasts become invested with a sporocyst, but there are as yet no signs of the sporozoites. After three days *four* rounded homogeneous bodies are budded off from the main granular mass of each sporoblast. At this stage the sporocysts are of an elongated ovoid shape with a lensiform thickening at the narrower pole. They measure about 9·6 × 8·0 μ. The rounded bodies are the precursors of the sporozoites (Fig. III, 9–12). Towards the fifth or sixth day they develop into four club-shaped sporozoites, about 7·6 μ long, arranged without any order within the sporocyst (Fig. III, 14, 15). In the course of the development of the sporozoites the sporocystic residue becomes considerably reduced in bulk. At this stage the oocyst usually ruptures and the sporocysts are found free in the medium, together with the empty cyst capsules (Fig. III, 13). The sporocyst when fully developed is more rounded than in the earlier stages, while the lensiform structure is practically obliterated, leaving only a thickening at the narrower pole.

The sporogony of this sporozoon thus results in the production of *sixteen sporozoites* divided between *four sporocysts*. This parasite therefore represents a new type of Coccidia characterised by *tetrazoic tetrasporocystid* oocysts, the status of which will be discussed below.

The study of the endogenous stages of development of this parasite brought disappointing results. The material consisted of various portions of the small intestine of the two infected snakes. In one of these a painstaking search through dozens of sections revealed but a few isolated stages of the parasite, while in the other no parasites could be found. The infection in both the snakes had evidently terminated with the discharge of the oocysts, leaving a few stragglers behind to complete their development in the intestinal tissues.

Legend to Fig. III.

Wenyonella africana gen.n., sp.n.

1–6. Endogenous stages (sections of small intestine): 1, schizont—first division; 2, schizont with dividing nuclei (reconstructed from 2 sections); 3, group of merozoites within host-cell cavity (cyst?) (reconstructed from 3 sections); 4–6, growing macrogametocytes (?). 7–15. Exogenous stages (sporogony) (from living specimens): 7, oocyst with unsegmented zygote; 8, four sporoblasts in oocyst; 9, 10, oocysts with four immature sporocysts; 11, 12, sporocysts (isolated artificially), each with four immature sporozoites budding off from sporoblast; 13, empty oocyst after escape of sporocysts; 14, 15, free mature sporocysts. (All the figures were drawn with the aid of a camera lucida. ×2000.)

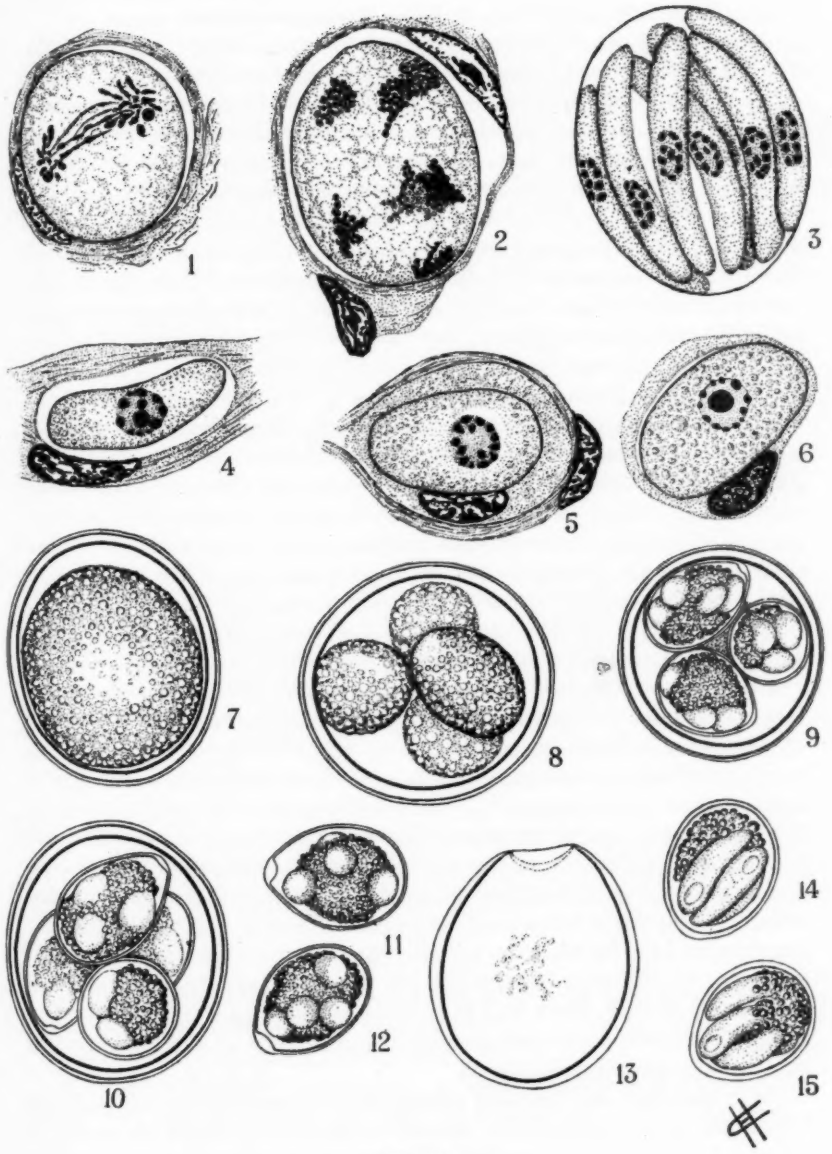

Fig. III.

The parasites were in the subepithelial tissues of the small intestine, within cells of the lamina propria of the villi. Practically the only forms observed were some stages of schizogony and, probably, macrogametocytes.

The dividing schizonts measure from 15·2 × 13·5 to 20·9 × 15·2μ (Fig. III, 1, 2). Division appears to be of the type described for some of the *Eimeria*, *i.e.* an atypical form of karyokinesis. Fig. III, 1, shows the first division of the schizont nucleus in late anaphase or early telophase. The karyosome is divided into two, the daughter elements, surrounded by chromatin strands (chromosomes?), having moved to the opposite poles. The total number of merozoites produced is probably about twenty, as estimated from combined sections. The merozoites are large sausage-shaped bodies, measuring about 20 × 3μ. Their nucleus is central, has an elongated shape, and appears to be composed of a small number of coarse chromatin granules. The bundle of merozoites is surrounded by a distinct membrane, but whether this is produced by the parasite or by the host cell could not be determined (Fig. III, 3).

The other stages seen were represented by elongated bodies of different dimensions. Some of them are sausage-shaped, measuring about 15 × 5μ, others are considerably broader, from 13·5 × 8·0 to 15·2 × 8·5μ (Fig. III, 4–6). The nucleus of these forms is of the vesicular type: there is a large round karyosome near the centre and a row of coarse chromatin granules along the periphery. The granules vary somewhat in size and some of them project into the space between the karyosome and the nuclear membrane. The cytoplasm is filled with globules which are especially bulky in the larger forms.

There are two possible interpretations of the nature of the forms just described. They may be young schizonts, representing the successive stages of growth from the merozoite to the ripe dividing schizont. On the other hand, they may be developing macrogametocytes. There is no direct evidence to show which alternative is the correct one. The points in favour of regarding these forms as macrogametocytes are their elongated form, the presence of numerous large granules (reserve matter?) in the cytoplasm, and the nuclear structure. The nucleus is typical of the macrogametocyte and, moreover, shows no preparatory signs of division, such as one would expect to see in a schizont of this size, judging by the dividing ones. Another argument in favour of the sexual nature of these stages is the evidence, mentioned above, that the parasite has already completed its life cycle, and that the few intracellular forms observed are stages, the development of which has for some reason been retarded. In that case it is more likely that they represent the stages immediately preceding the oocysts, *i.e.* the gametocytes, than that they are schizonts destined to repeat the asexual reproduction.

As is seen from the foregoing description, the parasite in question differs from all the other Coccidia in the structure of the mature oocyst and cannot be assigned to any known genus of these sporozoa. It is therefore necessary to create a new genus to comprise the tetrazoic tetrasporocystid Coccidia, and I propose to name it *Wenyonella* gen.n., with *Wenyonella africana* sp.n. (from

Boaedon lineatus) as type-species. The generic name is given in honour of Dr C. M. Wenyon, F.R.S.

The higher systematic group to which the new genus *Wenyonella* belongs cannot be established with absolute certainty, owing to the absence from the material available of some important stages of diagnostic value. The choice lies between the suborders Eimeriidea and Adeleidea[1]. In the former group the microgametocyte is not associated with the macrogametocyte before fertilisation and produces numerous gametes, while in the latter the male and female gametocytes associate in pairs (syzygy) and typically four microgametes are produced.

Unfortunately, no male forms could be found among the endogenous stages of development of this parasite. Though there is thus no exact criterion for the classification of the new genus, it shows a definite affinity to the Eimeriidea in a number of important features. It has already been pointed out that certain stages of the endogenous development of *Wenyonella africana* probably represent female gametocytes. If this interpretation is correct these forms should be regarded as belonging to the Eimeriidea, since they are not associated with a male gametocyte, as the corresponding stages of the Adeleidea. The new coccidium has also a number of morphological features peculiar to the Eimeriidea. Thus, its oocyst is a thick-walled double-layered capsule of a type which is quite common among the Eimeriidea, but unusual in the Adeleidea, in which the oocyst wall is generally very thin. Moreover, the general habitus of *Wenyonella* in all its stages of sporogony is similar to that of members of the genera *Eimeria* and *Isospora*. Thus, an *Eimeria* would become a *Wenyonella* if the number of sporozoites in each of its sporocysts were doubled, while an *Isospora* could be transformed into a *Wenyonella* by doubling the number of its sporocysts[2]. The fact that the oocyst of *Wenyonella* produces sixteen sporozoites is again a character suggestive of the Eimeriidea, since in all the Adeleidea, except *Chagasella*, the total number of sporozoites within the oocyst is indefinitely large. It should also be noted that among the true Coccidia of the suborder Adeleidea there is not a single form parasitic in vertebrates[3]. All these considerations make it highly probable that the new genus *Wenyonella* belongs to the suborder Eimeriidea, family Eimeriidae, to which it is provisionally referred.

Diagnosis.

Systematic position. *Wenyonella* gen.n.: Coccidiida Eimeriidae (?), producing tetrazoic tetrasporocystid oocysts.

Type-species: *Wenyonella africana* sp.n.

Description. Oocysts subspherical or ovoid: 18·5 × 16·0–19·2 × 17·6 μ; sporocysts ovoid, with lensiform "knob" at one pole: 9·6 × 8·0 μ; sporozoites

[1] According to the classification adopted by Wenyon (1926).

[2] Cf. Fig. VI, p. 383.

[3] *Klossiella*, parasitic in rodents, is an exception, but it is more closely allied to such haemococcidia as *Hepatozoon* than to the true Coccidia.

irregularly arranged; large sporocystic residue; sporulation time 5–6 days; unsegmented oocysts discharged from host.

Habitat. Small intestine (subepithelial) of *Boaedon lineatus* (Ophidia Colubridae).

Locality. Entebbe, Uganda.

(4) **Eimeria urnula** sp.n.

Fig. IV.

Six half-grown cormorants, *Phalacrocorax carbo lugubris*, were taken from nests in Ambatch trees near the shore of Victoria Nyanza, in the vicinity of Entebbe.

In the rectal and cloacal contents of one of these birds were found unsegmented oocysts of a species of *Eimeria*. The oocysts are roughly ovoid or piriform, but there is considerable individual variation in their shape, since the majority are markedly asymmetrical (Fig. IV, 4–9). The oocyst wall is thick and in the later stages of sporogony provided with a pseudomicropyle at the narrower pole. The dimensions of the oocysts vary from $17{\cdot}6 \times 12{\cdot}8$ to $23{\cdot}2 \times 13{\cdot}6\,\mu$. The zygote is a spherical body with numerous globular granules in its cytoplasm. Its diameter is about $12\,\mu$ (Fig. IV, 4).

The development of the parasite in chromic acid proceeds as follows. After one day four rounded sporoblasts are produced (Fig. IV, 5, 6). There is no oocystic residue, but sometimes from one to three small globules are visible within the oocyst: these may, however, be present before the zygote has divided and cannot therefore be regarded as true residual bodies. After two days the sporocyst is formed around the sporoblasts, and on the third day two fully developed sporozoites are produced in each sporocyst.

The mature sporocysts (Fig. IV, 7–9) measure about $10{\cdot}4 \times 5{\cdot}6\,\mu$. They are elongated ovoid or ellipsoid; the sporocyst wall is thin, with a lensiform thickening at the narrower pole. The two sporozoites, which are about $8{\cdot}4\,\mu$ long, are sausage-shaped bodies, both ends of which are similar. They are slightly curved and lie facing each other with their concave surfaces separated by a fairly large rounded sporocystic residue.

One of the most characteristic features of the oocyst of this coccidium is the pseudomicropyle. In the zygote stage—sometimes later—the oocyst wall is of uniform thickness all round (Fig. IV, 4). By the time the sporoblasts are produced there appears a circular "suture" at the narrower pole (Fig. IV, 6). Later, when sporogony is completed, the area limited by the "suture" becomes markedly thinner than the rest of the wall, and the whole oocyst now looks like a kind of urn or vase covered with a saucer-shaped lid (Fig. IV, 7, 9). There can be little doubt that the contents of the oocyst ultimately emerge at this part of the wall, which appears to be the point of least resistance. It could not be established, however, whether the operculum-like structure actually represents a lid comparable to the operculum of some trematodes and

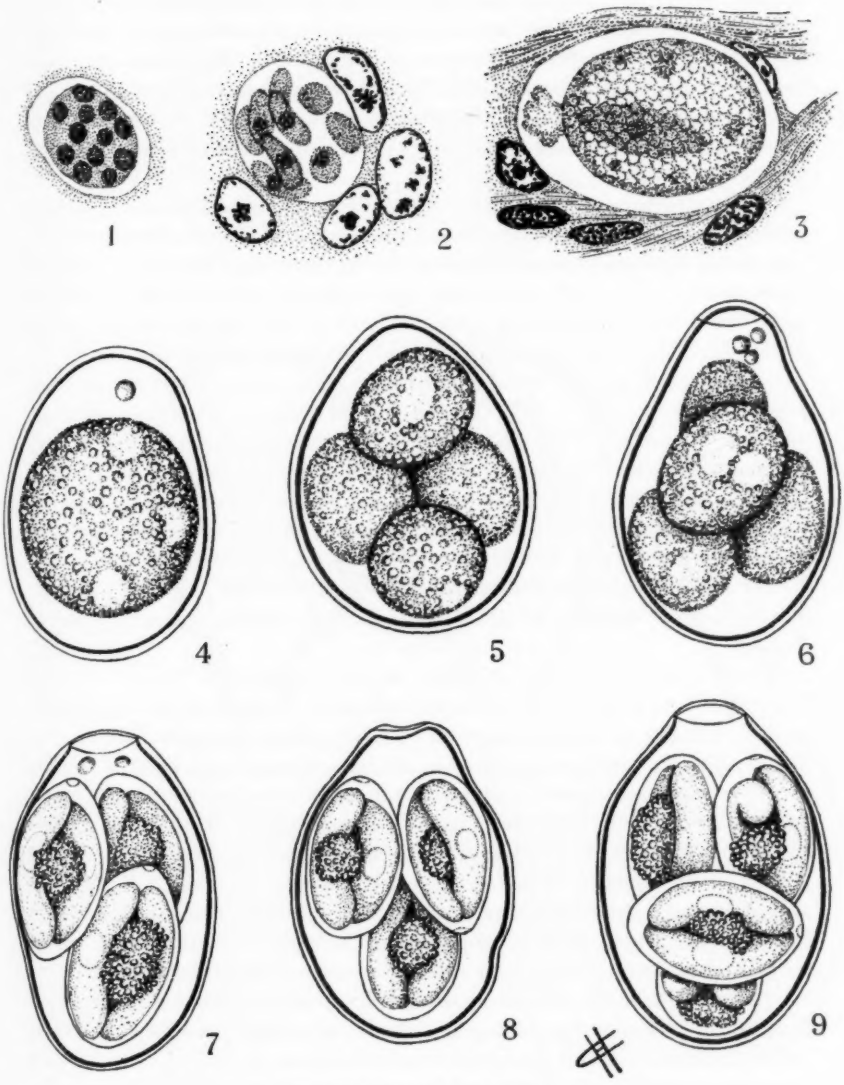

Fig. IV. *Eimeria urnula* sp.n.

1–3. Endogenous stages (sections of small intestine): 1, schizont; 2, merozoites; 3, female gametocyte or gamete. 4–9. Exogenous stages (sporogony) (from living specimens): 4, oocyst with unsegmented zygote; 5, 6, oocysts with four sporoblasts (in 6 differentiation of pseudomicropyle initiated); 7–9, oocysts with mature sporocysts (pseudomicropyle fully developed; in 8 collapsed). (All the figures were drawn with the aid of a camera lucida. ×2500.)

detachable as a whole, or simply an attenuated dome-shaped area of the oocyst wall. In the former case the operculum would not be continuous with the rest of the wall and the limiting line would be a real suture or fissure, while in the latter case it would represent the border-line between the thick-walled main part of the oocyst and the thin-walled apical area, the wall being continuous in both parts. This structure, *i.e.* the attenuated polar area of the oocyst, corresponding in position and function to the micropyle, might be called the pseudomicropyle.

Various portions of the intestine of the cormorant were fixed and examined in sections. Stages of the endogenous development of the parasite occurred in very small numbers in the epithelium and in the subepithelial tissues of the small intestine. The only forms seen were schizonts and a female form. The schizonts are small, measuring about $7{\cdot}5 \times 5{\cdot}5\,\mu$ and showing about a dozen nuclei (Fig. IV, 1). The merozoites are short, sausage-shaped bodies (Fig. IV, 2). The only other stage seen was evidently a macrogamete or a recently fertilised zygote, measuring $11{\cdot}5 \times 9{\cdot}5\,\mu$ (Fig. IV, 3). Its nucleus is drawn out, with one end near the surface of the body (fertilisation spindle); the cytoplasm is very granular and contains a number of dark staining inclusions (reserve material).

Up to the present only one coccidium has been recorded from cormorants, by Labbé (1896). This parasite, named *Coccidium roscoviense*, but now known as *Eimeria roscoviensis*, was found in *Phalacrocorax aristotelis*[1] at Roscoff, in France. According to the author (Labbé, 1893, 1896, 1899), the same parasite occurs in other birds (*Charadrius, Arenaria, Numenius, Totanus, Calidris, Tringa*).

Eimeria roscoviensis has piriform oocysts, measuring $16–18 \times 14–16\,\mu$. The oocyst "est tronquée à l'extrémité antérieure formant là, par l'amincissement de sa paroi, un pseudomicropyle" (Labbé, 1893). The sporocysts are also pear-shaped. The early description was not accompanied by an illustration, but in 1896 a figure of a mature oocyst of this coccidium was given. From this figure it is possible to supplement Labbé's account and to define the characters of *E. roscoviensis* as follows: Oocyst piriform, narrower pole slightly drawn out ("neck") and flattened at the top (truncated); at this point the wall is attenuated, forming a pseudomicropyle (cf. Fig. V, 3). Sporocysts piriform, with knob-like thickening at the narrower pole. Two curved sporozoites lying side by side, their concave surfaces in contact with the sporocystic residue.

The parasite of the African cormorant differs in the structure of its oocyst from all the other species of *Eimeria* described from birds. There are, however, certain features in which the present form resembles *E. roscoviensis*. In both the oocyst is roughly piriform and its narrower pole is differentiated into a kind of pseudomicropyle represented by a wide aperture (micropyle?) covered over by a thin-walled area. This covering is dome-shaped in the *Eimeria* of the

[1] Labbé (1896, 1899) referred to this cormorant or "shag" as *P. graculus* and *P. cristatus*. Dr G. Carmichael Low has kindly informed me that the correct name for this bird is *P. aristotelis*, the ones given by Labbé being its synonyms.

African cormorant and flat in that of the European one (Fig. V, 3 and 5). The flat lid-like structure gives the oocyst of *E. roscoviensis* its truncated appearance, in which it differs from that of our parasite. Apart from this the oocysts of the two forms differ in size. There is a more pronounced difference between the sporocysts of these parasites. In *E. roscoviensis* they are definitely piriform, whereas in the new *Eimeria* they are rather ellipsoid. In the former parasite the sporozoites are parallel to each other and lie on one side of the sporocyst, with the residue on the opposite side, whereas in our parasite they face each other, with the residue between them.

The differences noted above are sufficiently marked to recognise the coccidium of the African cormorant as a distinct species which I propose to name *Eimeria urnula* sp.n.

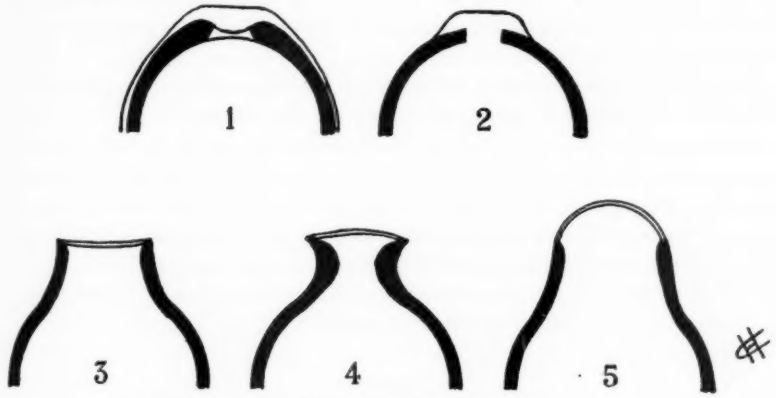

Fig. V. *Diagrams illustrating polar structures in Eimerian oocysts.*

1, *Eimeria stiedae:* micropyle covered by thickening of outer oocyst wall; 2, *E. truncata:* micropyle covered by "cap"; 3, *E. roscoviensis:* pseudomicropyle (=attenuated polar area of oocyst) flat, "neck" not pronounced; 4, *E.* (=*Jarrina*) *paludosa:* pseudomicropyle flat (?), definite "neck" present; 5, *E. urnula* sp.n.: pseudomicropyle dome-shaped. [Figs. 1–4 adapted from Reichenow (1929), Lerche (1923), Labbé (1896), and Léger and Hesse (1922) respectively.]

Some time ago Léger and Hesse (1922) created the genus *Jarrina* for Coccidia possessing all the characters of *Eimeria*, but differing from the typical forms in the structure of the oocyst. In the type-species *J. paludosa*, one of the poles of the oocyst is raised in the form of a short "neck" (Fig. V, 4), and the wall is punctated. The authors included in this genus *Eimeria roscoviensis* (Labbé, 1893) and *E. truncata* (Railliet and Lucet, 1891). Except that in Léger and Hesse's form the "neck" is more pronounced, the similarity of the oocyst of *Jarrina paludosa* to that of *Eimeria roscoviensis* is undeniable, while the oocyst of the new species, *E. urnula*, is a variation of the same type.

As regards *E. truncata*, the latest observations by Lerche (1923) have shown that the oocyst of this parasite represents an ovoid capsule with a wide aperture (micropyle) at the narrower pole. This is sometimes surmounted by

a flat-topped "cap" which extends beyond the limits of the aperture (Fig. V, 2). There is, thus, no structure comparable to the "neck" of *Jarrina*. It must be noted, however, that Lerche's paper appeared later than that of Léger and Hesse. These authors based their comparison upon the original description of *Eimeria truncata* (*Coccidium truncatum*) given by Railliet and Lucet (1890, 1891). According to the last-named authors, the oocyst of *E. truncata* is slightly raised and truncated at the end where the micropyle is situated. This would seem to agree with the appearance of the oocyst of *Jarrina*, but Railliet and Lucet also state that the oocyst of their coccidium is like that of *Eimeria stiedae*. Now in the latter there is a wide micropyle in the inner wall of the oocyst, covered over by a flat cap-like local thickening of the outer wall (Fig. V, 1), and the whole structure is very similar to that of *E. truncata*, as described by Lerche (1923). Thus, on whichever authority the comparison is based, it is evident that the oocyst of *E. truncata* is of a different type from that of *Jarrina paludosa*.

The oocyst structures referred to above are represented diagrammatically in Fig. V, showing at a glance the characters noted in the text.

As regards the status of *Jarrina* itself, with the single species *J. paludosa*, its separation from *Eimeria*, either as a genus or subgenus, on the grounds of a peculiar structure of the oocyst, cannot be justified unless new genera are created for all the other species differing from each other in this particular. This would render the classification of these Coccidia too unwieldy, since the structure of their oocysts varies considerably. In view of these facts I propose to retain *Jarrina* in the genus *Eimeria sensu lato*.

Diagnosis.

Systematic position. *Eimeria urnula* sp.n. (Coccidiida Eimeriidae).

Description. Oocysts ovoid or piriform, asymmetrical, with wide pseudo-micropyle at narrower pole: $17{\cdot}6 \times 12{\cdot}8$–$23{\cdot}2 \times 13{\cdot}6\,\mu$; sporocysts ovoid or ellipsoid, with lensiform "knob" at one pole: $10{\cdot}4 \times 5{\cdot}6\,\mu$; sporozoites facing each other and separated by sporocystic residue; unsegmented oocysts discharged from host.

Habitat. Small intestine (intra- and subepithelial) of *Phalacrocorax carbo lugubris* (juv.) (Aves Pelecaniformes).

Locality. Victoria Nyanza, near Entebbe, Uganda.

III. Classification of the Eimeriidea.

The systematics of the Coccidia has undergone many changes in adapting itself to the gradually increasing knowledge of this group. Since this knowledge is still very incomplete there is no generally accepted system of classification, all the existing ones being provisional. They differ from each other both in the criteria upon which the systematic units are based, and in the nomenclature adopted for their designation.

It is generally admitted that the composition of the mature oocyst, viz. the number of sporocysts and sporozoites it contains, provides the most convenient diagnostic characters for the differentiation of the coccidian genera. The division of the true Coccidia into two major groups, the suborders Eimeriidea and Adeleidea (*sensu* Wenyon, 1926), distinguished by the type of the fertilisation process, is equally well established at present. Opinions are divided, however, regarding the basis upon which the genera should be united into the intermediate systematic units, such as subfamilies or families.

One school, represented by Minchin (1912), Reichenow (1921) and Wenyon (1926), follows Lühe (1906) in arranging the genera into higher groups mainly according to the type of schizogony. Another school adopts the characters of the ripe oocyst as the basis for the classification of the genera. The most important representatives of this school are Schneider (1881) and Léger (1900, 1911). These two authors differ, however, in the choice of the characters according to which the genera are grouped. While Schneider and his followers (Bütschli, 1882; Labbé, 1899; Minchin, 1903; and some others) arranged them according to the *number of sporocysts* within the oocyst, Léger and his adherents (Mesnil, 1903; Poche, 1913; Doflein, 1916; Pinto, 1928; and others) classified them according to the *total number of sporozoites* in the oocyst.

An ideal system of classification is one constructed on a phylogenetic basis, in which the natural affinities of the constituent members are taken into consideration, and these are arranged in the ascending order, from the more primitive forms to the more highly organised ones. Unfortunately, in the case of the Coccidia, this ideal is unattainable, on account of the incompleteness of our knowledge of this group, especially as regards the endogenous stages of development.

In practice the determination of the Coccidia is based on the characters presented by the mature oocyst, which are constant and well defined. The quantitative differences in these characters (number of sporocysts and sporozoites) are of generic value, while the qualitative differences (cytological details of structure) are of specific value. The isolated stages of the endogenous development in the various genera cannot serve for the identification of these parasites, since they do not show any distinctive peculiarities. In view of these facts it would appear that—at present at least—the oocyst characters of the Coccidia afford more constant and definite criteria for the arrangement of the genera into higher systematic units than their asexual stages of development. Apart from this practical aspect of the question, the fact that the variations in the structure of the mature oocyst, characteristic of the different genera, are definitely correlated and show a regular gradation, renders it highly probable that a classification based on these characters is also a natural one.

The most recent system of classification on these lines is that of Léger (1911), if we disregard later modifications of his system which differ from the original one chiefly in the nomenclature. Léger divides the Eimeriidea (which is the only group with which we are concerned here) into the following three

groups, according to the *total number of sporozoites* in the oocyst: *Tetrazoica*, *Octozoica* and *Polyzoica*[1].

To these three groups could now be added a new one, *Heccaidecazoica*, characterised by the production of a total number of *sixteen* sporozoites in the course of sporogony. The new group should occupy a position between Léger's *Octozoica* and *Polyzoica*. This addition is necessitated by the discovery of two new types of Eimeriidea: one, *Dorisiella scolelepidis* Ray 1930, in which there are *sixteen sporozoites* divided between *two sporocysts*; the other, *Wenyonella africana* gen.n., sp.n. (described in the present paper), in which the oocyst contains *sixteen sporozoites* divided between *four sporocysts*.

It is necessary to make a few remarks with regard to *Dorisiella*. The entire development of this parasite takes place within the epithelial cells of the gut of its host, a polychaete worm, *Scolelepis fuliginosa*. The zygote of this coccidium is said to give rise to two sporocysts without the formation of an oocyst. The author (Ray, 1930, p. 476) states that "there is no common envelope or oocyst, but the two sporoblasts lie in the same vacuole of the epithelial cell." When fully developed the *isolated* sporocysts escape from the host cells into the lumen of the gut.

The conditions under which this parasite develops are not unlike those described above for *Isospora dirumpens* (p. 364) and for other members of this genus, completing their sporogony within the host tissues and escaping into the lumen of the gut in the form of free sporocysts. In all such parasites the oocyst wall is extremely delicate and difficult to detect. In the case of *I. dirumpens* the presence of an oocyst could only be established accidentally in a few isolated specimens, while in sections of the gut the thin oocyst is practically invisible, only the thick-walled sporocysts standing out clearly[2]. In reading Ray's account of *Dorisiella scolelepidis* it occurred to me that his parasite might possibly exhibit the same peculiarities of structure as the species of *Isospora* referred to above, since its mode of development is similar. An examination of the figures confirmed my suspicions. Fig. 17, Pl. XLI, of the paper in question (Ray, 1930) shows clearly *two lines* connecting the two sporoblasts. There can be no doubt that these lines represent the thin oocyst wall moulded tightly around the sporoblasts, exactly as in *Isospora dirumpens* (cf. Fig. II, p. 367). Probably the oocyst of *Dorisiella* ruptures very early and its presence is, therefore, easily overlooked.

Between the groups created by Léger there can be observed a certain

[1] It should be noted here that Léger's terms, *Tetrazoica*, *Octozoica*, etc., indicate the total number of sporozoites *in the oocyst*, whereas in the terminology more commonly used the adjectival form of these names (tetrazoic, etc.) denotes the number of sporozoites *within each sporocyst*. Since the use of these terms in both senses is unavoidable in the discussion which follows—in view of the frequent references to Léger's classification—care has been taken to avoid ambiguity in the general context, and, furthermore, these terms are written in *italics* when denoting the number of sporozoites in the oocyst, and in ordinary type when referring to the number in each sporocyst.

[2] In the illustrations (Fig. II, 1 and 2), the oocyst wall stands out more sharply than in the actual sections.

parallelism. Thus, the asporocystid[1] and disporocystid forms of his *Tetrazoica* (*Cryptosporidium*, *Cyclospora*) are represented by homologous forms in the *Octozoica* (*Pfeifferinella*, *Isospora*), while the tetrasporocystid *Eimeria* in the latter group has its homologue in *Angeiocystis* of the *Polyzoica*. The homologous forms differ from each other only in the number of sporozoites within each sporocyst.

The parallelism already evident in Léger's classification is further enhanced by the addition of the new group, *Heccaidecazoica*, in which *Dorisiella* and *Wenyonella* are homologous to *Isospora* and *Eimeria* respectively.

These relations can be appreciated at a glance, if all the known genera of the Eimeriidea (excluding the atypical *Selenococcidium*) are represented graphically and arranged in parallel groups, according to their oocyst characters, as shown in Fig. VI.

In this diagram the four vertical columns (A, B, C, D) correspond to Léger's groups, with the addition of the new *Heccaidecazoic* one and certain modifications in his *Polyzoic* group to be mentioned later. If we take the most complete group, the *Octozoic*, it will be seen that it comprises four structural types of oocyst, characterised as follows: (1) Sporocysts absent (**Asporocystid**), eight sporozoites within oocyst (*Schellackia*, *Pfeifferinella*); (2) one sporocyst (**Monosporocystid**) containing eight sporozoites (*Caryospora*); (3) two sporocysts (**Disporocystid**) containing four (= 8/2) sporozoites each (*Isospora*); (4) four sporocysts (**Tetrasporocystid**) containing two (= 8/4) sporozoites each (*Eimeria*). The structural types of the *Octozoic* group have their homologues in the other groups. In the diagram the genera representing these types are accordingly placed in line with the homologous genera of the *Octozoic* group. After all the genera are arranged in this manner there are left a certain number of gaps in the various groups, indicating structural types of the oocyst not represented by any known Coccidia. Since, however, the structure of the forms which might occupy the vacancies is determined by those immediately above and below the gaps in the vertical groups (Fig. VI, A, C, D), these forms are inserted in the appropriate places as hypothetical ones (shown by interrupted circles in the diagram). Future discoveries might show whether there actually exist Coccidia bearing the characters of these hypothetical forms. It may be pointed out that in the meantime some of the gaps which existed in Léger's original system of classification (Fig. VI, 3*C*, 4*C* and 1*D*) have already been filled by *Dorisiella* and *Wenyonella*, in the new *Heccaidecazoic* group, and by *Lankesterella* in the *Polyzoic* group.

The *Polyzoic* group (D) in the present scheme does not correspond exactly to Léger's *Polyzoica*, which comprises polysporocystid as well as tetrasporocystid Coccidia (*Barroussia*[2], *Caryotropha*, *Angeiocystis*). In the present

[1] Léger refers to the asporocystid Coccidia as monosporocystid ("monosporées"), for reasons stated below.

[2] This genus is commonly spelt *Barrouxia*, though it was originally named *Barroussia* by Schneider (1885). I follow Poche (1913) in adopting the original version, since improvements in the orthography of a scientific name cannot be accepted, according to the Rules of Zoological Nomenclature, in spite of the fact that *Barroussia* was "dédié à M. Barroux" (Schneider, 1885).

diagram the *Polyzoic* group (D) is made to agree in composition with the other three groups (A, B, C), while the polysporocystid Coccidia, which are not correlated with the three groups mentioned, are separated as an independent group (Fig. VI, 5).

The groups corresponding to Léger's classification in the diagram (Fig. VI, A, B, C, D) are not the only ones showing a definite relationship to each other: there is also a regular gradation of the generic characters in members of the horizontal rows (Fig. VI, 1, 2, 3, 4). In fact, the scheme shown in Fig. VI represents a kind of "Periodic System" of Classification of the Eimeriidea, the genera of which are arranged in horizontal and vertical rows in the ascending order of the number of structural elements contained in their oocysts.

Horizontally they form four rows of homologous series, differing from each other in the *number of sporocysts* within the oocyst, (1) **Asporocystid**, (2) **Monosporocystid**, (3) **Disporocystid**, and (4) **Tetrasporocystid**; in each of these rows or series the members (= genera) differ from each other in the *number of sporozoites* within each sporocyst, this number being equal to that of the preceding genus multiplied by 2.

Vertically the genera fall into the four groups already referred to (A, *Tetrazoic*, B, *Octozoic*, C, *Heccaidecazoic* and D, *Polyzoic*) which differ from each other in the *total number of sporozoites* within the oocyst, while the individual members of each vertical group are distinguished from each other in the *number of sporocysts* between which the sporozoites are divided, this number being equal to that of the preceding genus multiplied by 2.

It is thus seen that in this diagram the oocyst characters of each member (= genus) are determined by those of its neighbours in the vertical and horizontal rows. A glance at the diagram will show that it is incomplete in several respects. It is obvious that at the bottom of the vertical rows there is room for more structural types. These can be derived from the last ones in the rows by multiplying the number of sporocysts they contain by 2 and dividing the sporozoites in equal numbers between them, until a type containing *n* sporocysts with *one* sporozoite each is reached, as shown in group A. Since oocysts with these characters represent the ultimate type in each vertical row, it follows that each row has one member more than the preceding one, on its left.

The Polyzoic group (D), in which the exact number of sporozoites is unknown, is a *collective group*. As regards the Polysporocystid *polyzoic* forms

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Legend to Fig. VI.

Genera of the Family Eimeriidae, arranged according to the characters of their mature oocysts.

Groups A, B, C, D correspond to Léger's classification; groups 1, 2, 3, 4, 5 are those adopted for the present classification.

The diagrams represent mature oocysts characteristic of the different genera. Complete circles refer to existing types, while interrupted ones denote hypothetical forms (Vacant). The heavily drawn circles indicate *oocysts*; the finely drawn ones *sporocysts*. The sporozoites are represented by the curved or rod-shaped bodies.

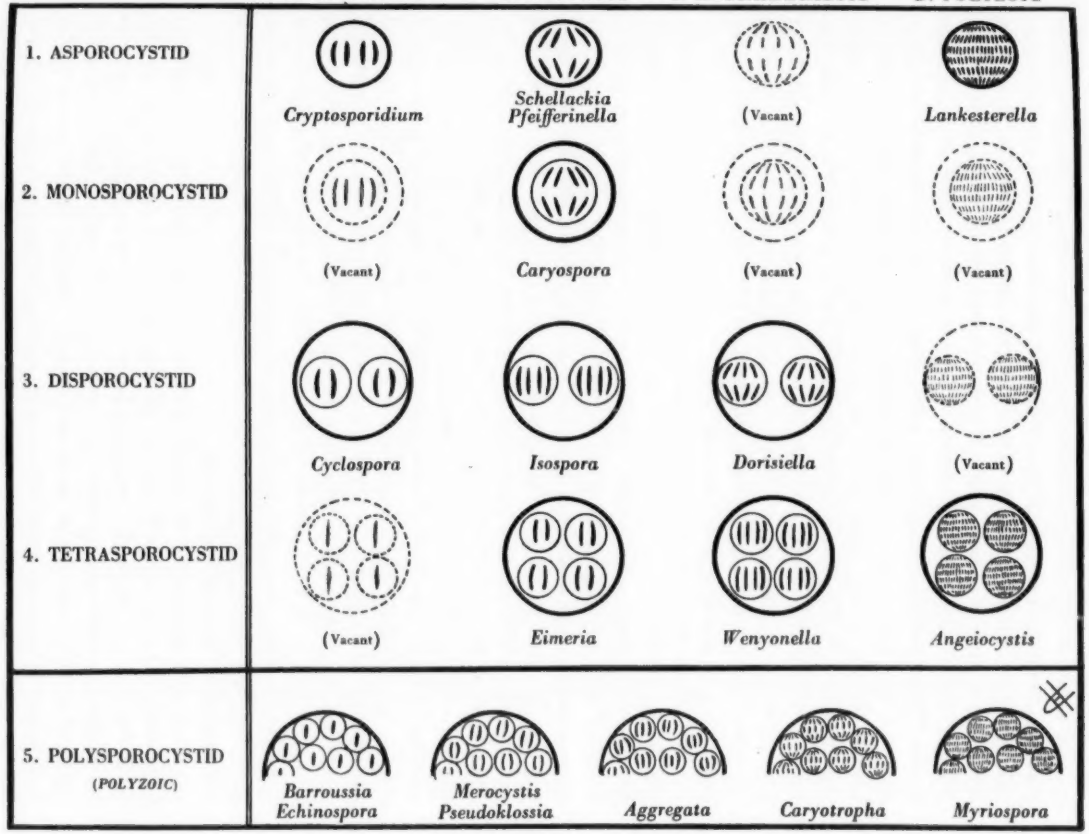
A. TETRAZOIC
B. OCTOZOIC
C. HECCAIDECAZOIC
D. POLYZOIC
1. ASPOROCYSTID
Cryptosporidium
Schellackia Pfeifferinella
(Vacant)
Lankesterella
2. MONOSPOROCYSTID
(Vacant)
Caryospora
(Vacant)
(Vacant)
3. DISPOROCYSTID
Cyclospora
Isospora
Dorisiella
(Vacant)
4. TETRASPOROCYSTID
(Vacant)
Eimeria
Wenyonella
Angeiocystis
5. POLYSPOROCYSTID
(POLYZOIC)
Barroussia Echinospora
Merocystis Pseudoklossia
Aggregata
Caryotropha
Myriospora

Fig. VI.

(Fig. VI, 5), it is possible that some of them with dizoic sporocysts (*e.g. Merocystis, Pseudoklossia*) represent the penultimate members of a vertical *Polyzoic* group (D), while others (*e.g. Barroussia, Echinospora*), having monozoic sporocysts, might represent the ultimate type of such a group.

The arrangement of the coccidian genera shown in Fig. VI can serve as the basis of a formal classification of the Eimeriidea *according to the structure of the mature oocyst.* The two methods of grouping the genera—(*a*) in four horizontal homologous rows, characterised by the number of sporocysts (1, 2, 3, 4), and (*b*) in four vertical rows characterised by the total number of sporozoites (A, B, C, D)—indicate two possible methods of classification both of which are not new. The former (*a*) would be a modification and amplification of Léger's system (Léger, 1911), while the latter (*b*) would revive some of the earlier classifications, especially that of Minchin (1903), who divided the Coccidia into the following four families: (1) Asporocystidae, (2) Disporocystidae, (3) Tetrasporocystidae, (4) Polysporocystidae. Though the principle on which Minchin's classification is based corresponds to the present grouping, it is needless to say that it is antiquated in every respect.

Of the two possible classifications preference should be given to the one which appears to be more "natural," *i.e.* in which the affinities of its members are more accurately represented. Unfortunately, this must remain an open question at present, since both classifications appear to be equally plausible when interpreted on these lines. Thus, if the *Octozoic* group (Fig. VI, B) is taken as a representative of Léger's classification, it may be argued that its members are related as follows: the monosporocystid form (2, B) originated from an asporocystid one (1, B) by enclosing the eight sporozoites within a sporocyst; the disporocystid form (3, B) originated from the latter by duplication of the number of sporocysts each of which took half the number of sporozoites; and finally, by a further duplication of the number of sporocysts with an equal distribution of the sporozoites, the tetrasporocystid form (4, B) was produced. There appears to be equal justification for regarding the horizontal homologous rows (Fig. VI, 1, 2, 3, 4) as natural groups. Here the number of sporocysts is constant for each group, but the number of sporozoites within each sporocyst increases regularly in each genus from left to right and may have evolved from the preceding type by duplication.

Since the relative merits of the two methods of classification cannot be estimated on the grounds of the natural affinities, which appear to be equally well founded in both cases, preference should be given to practical considerations. In my opinion, a classification based primarily on the number of sporocysts within the oocyst, and secondarily on the number of sporozoites within each sporocyst, is a more rational one than that proposed by Léger (1911), and has the advantage of greater simplicity and uniformity, since each of its groups (Fig. VI, 1, 2, 3, 4) comprises homologous types (= genera) differing from each other only in one particular—the number of sporozoites in each sporocyst—whereas in Léger's classification each group (Fig. VI, A, B, C, D) is composed

of types differing from each other both in the number of sporocysts and in the number of sporozoites each of them contains. Léger regards the production of sporocysts as a phenomenon of secondary importance because sporozoites have occasionally been observed to develop without sporocysts in the oocysts of Coccidia normally provided with these structures. Thus, assuming that the sporocysts represent a variable character, while the total number of sporozoites in the oocyst is constant, Léger bases his classification primarily on the latter character[1]. Since, however, the disappearance of the sporocyst in *Caryospora simplex*, which is the example described by Léger (1911), was due to sporulation under septic conditions, it is an abnormality, and cannot, therefore, serve to support the author's contention.

Table III. *Classification of the Coccidiida Eimeriidea.*

	Diagnostic characters
Suborder EIMERIIDEA (Poche, 1913)	
I. Family SELENOCOCCIDIIDAE Poche 1913	
Genus *Selenococcidium*	
II. Family EIMERIIDAE (Minchin) Poche 1913	
1. Subfamily Cryptosporidiinae subf.n. .....1.	Oocyst ASPOROCYSTID
Genus *Cryptosporidium*	„ *Tetrazoic*
„ *Pfeifferinella* }	„ *Octozoic*
„ *Schellackia* }	
„ *Lankesterella*	„ *Polyzoic*
2. Subfamily Caryosporinae Wenyon 1926 .....2.	Oocyst MONOSPOROCYSTID
Genus *Caryospora*	Sporocyst *Octozoic*
3. Subfamily Cyclosporinae Wenyon 1926.....3.	Oocyst DISPOROCYSTID
Genus *Cyclospora*	Sporocysts *Dizoic*
„ *Isospora*	„ *Tetrazoic*
„ *Dorisiella*	„ *Octozoic*
4. Subfamily Eimeriinae Wenyon 1926 .....4.	Oocyst TETRASPOROCYSTID
Genus *Eimeria*	Sporocysts *Dizoic*
„ *Wenyonella*	„ *Tetrazoic*
„ *Angeiocystis*	„ *Polyzoic*
5. Subfamily Barroussiinae (Wenyon 1926) .....5.	Oocyst POLYSPOROCYSTID
Genus *Barroussia* }	Sporocysts *Monozoic*
„ *Echinospora* }	
6. Subfamily Aggregatinae subf.n.	
Genus *Merocystis* }	Sporocysts *Dizoic*
„ *Pseudoklossia* }	
„ *Aggregata*	„ *Trizoic*
„ *Caryotropha*	„ *Dodecazoic*
„ *Myriospora*.....	„ *Polyzoic*

I propose to divide the suborder Eimeriidea into two families: (1) Selenococcidiidae, and (2) Eimeriidae. The latter family, comprising genera with the characters indicated in the diagram (Fig. VI), is subdivided in accordance with the views expressed above, into 5 groups: four corresponding to the homologous series (Fig. VI, 1, 2, 3, 4) and one comprising the collective polysporocystid group (Fig. VI, 5). The names of these groups—as given in the diagram—though accurately defining their diagnostic features, cannot be employed for the higher systematic units, according to the Laws of Nomenclature. On account of this the four homologous groups (Fig. VI, 1, 2, 3, 4) have been

[1] This is also the reason why Léger ignores the absence of a sporocyst in the asporocystid genera *Cryptosporidium* and *Pfeifferinella*, and refers to them as "monosporées."

designated as subfamilies with the names taken from the typical genera. As regards the polysporocystid group (Fig. VI, 5), it is divided into two subfamilies: the Barroussiinae, which show closer affinities with the four homologous subfamilies, and the Aggregatinae. The proposed classification is set forth in Table III (p. 385) together with the diagnostic characters of each subfamily and genus. In this form the table can serve as a key.

The classification outlined above is admittedly provisional—like all the previous ones. It is proposed because it brings more order into the systematics of this group and facilitates the identification of the Coccidia. It should be emphasised, however, that the graphic scheme of classification in Fig. VI demonstrates the mutual relations of these sporozoa more accurately than the formal classification, and it, moreover, gives due prominence to the alternative claims of Léger's system, allowing of a compromise between the two methods of classification.

IV. Summary.

This paper contains an account of four new Coccidia from Uganda: *Caryospora legeri* sp.n., from a snake, *Psammophis sibilans*; *Isospora dirumpens* sp.n., from a snake, *Bitis arietans*; *Wenyonella africana* gen.n., sp.n., from a snake, *Boaedon lineatus*; and *Eimeria urnula* sp.n., from a bird, *Phalacrocorax carbo lugubris*. *Eimeria dukei* Lavier 1927, is recorded from a new host, *Chaerephon* (= *Nyctinomus*) *limbatus* (bat).

The characters of the new Coccidia are summarised in the diagnoses (pp. 364, 369, 373, 378). There is a list of all the known species of *Isospora* found in snakes and lizards. It is noted that *I. coelopeltis* Galli-Valerio 1926, is actually an *Eimeria*, and its name is amended to *E. coelopeltis*. The diagnostic characters of the three species of *Caryospora* are given in tabular form.

The discovery of a new type of Coccidia, with oocysts containing *sixteen sporozoites*, divided between *two sporocysts* in *Dorisiella* Ray 1930, and between *four* in *Wenyonella* gen.n., necessitated a revision of the systematics of the Eimeriidea.

When the genera comprising this suborder (with a few exceptions) are arranged in the ascending order of the number of structural elements (sporocysts and sporozoites) contained in their oocysts, they form parallel series or groups in the horizontal and vertical directions, which consist of homologous members, as represented graphically in Fig. VI (p. 383). The oocyst characters of each genus are determined by those of its four neighbours in the horizontal and vertical groups.

This scheme serves as the basis for the new classification proposed, which is represented in Table III (p. 385). The higher systematic units (subfamilies) are distinguished from each other by the number of sporocysts within the oocyst, while the genera of each subfamily differ from each other in the number of sporozoites within each sporocyst, thus forming a homologous series.

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COCCIDIA OF THE FELIDAE.

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(With 13 Figures in the Text.)

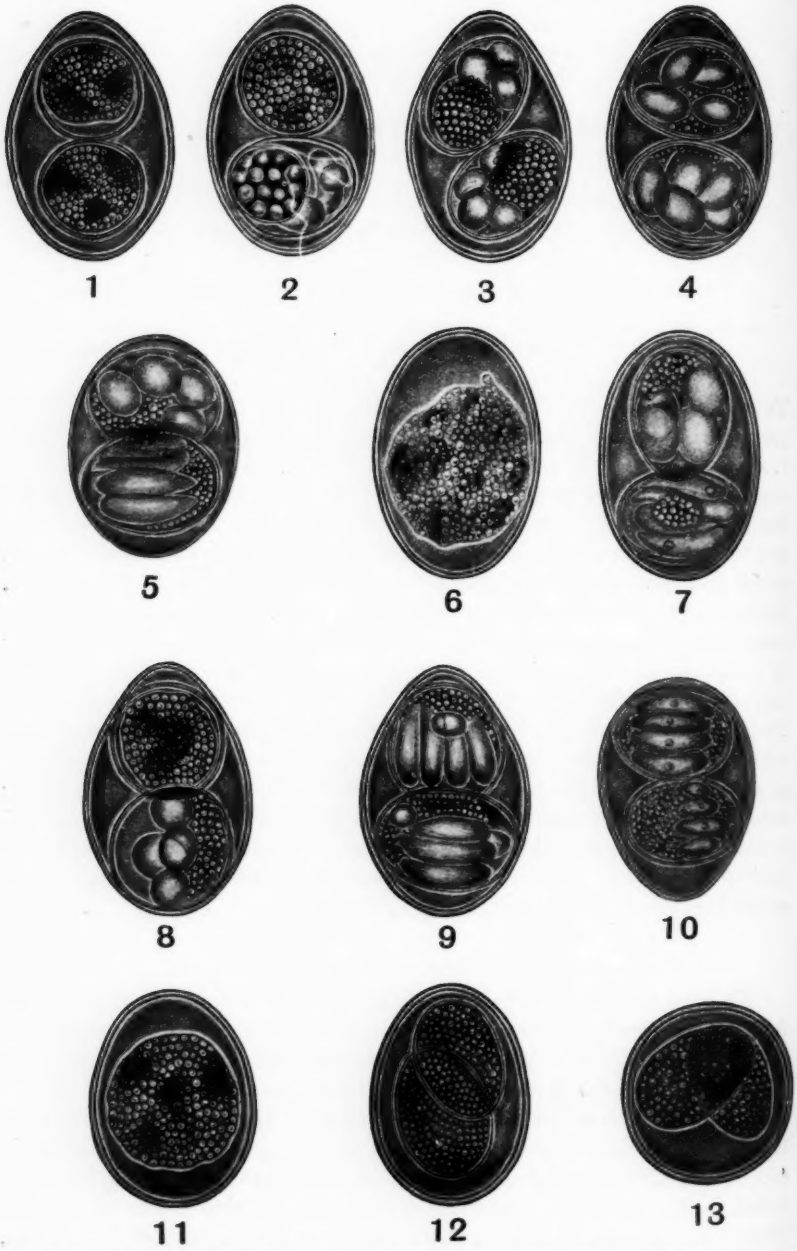
The first member of the Felidae in which coccidia were observed is the domestic cat, which harbours three species: *Isospora bigemina* var. *cati* (Stiles 1891), *I. rivolta* (Grassi 1879), and *I. felis* Wenyon 1923. Some of these have also been recorded from wild species of Felidae. In the faeces of two lion cubs at the Berlin Zoological Gardens, Möller (1923) found oocysts with one end rounded and the other pointed and possessing a micropyle; two spores developed in each oocyst and there was no residual body; the dimensions were 36–48 × 28–36[1]. Wenyon (1926) considers this coccidium to be *I. felis*. Rastegaïeff (1930) identified as *Eimeria felina*, oocysts without a micropyle, found in the faeces of a lioness at the Leningrad Zoological Gardens. The oocysts measured 29·7 × 16·2; he also found two species of coccidia in a tiger at the same place. One, named *Eimeria novowenyoni*, had rounded oocysts 14·4–18·9 in diameter, containing four spores; the other species had oviform oocysts with a micropyle at the pointed end; this form measured 22·7 × 14·4; the oocysts could not be cultivated and the parasite was provisionally named (genus?) *hartmanni*. Triffitt (1927) found coccidia in two Felidae at the Gardens of the Zoological Society of London. A lynx contained oocysts identified as *I. felis*, and an ocelot harboured a form identified as *I. rivolta*.

Personal observations.

We have examined ten individuals belonging to five species of Felidae as follows: two lions and a lioness, two tigers, a leopard, three lynxes and a jungle cat. These animals were either in the Leningrad Zoological Gardens or in a menagerie at Gandscha, and all harboured *Isospora* as follows:

The lions (*Felis leo*) harboured oocysts (Figs. 1–4) of which 75 derived from the lioness measured 37·8–50·4 × 28·8–36, averaging 44·5 × 33·6, being mostly 46·8 × 32·4 with form-index 1:065–0·83, average 1:0·71. The oocysts, cultivated in 2·5 per cent. potassium bichromate, developed two sporoblasts 18–21·6 in diameter, containing short oval spores averaging 27·9 × 18 in size. Each spore develops into four sporozoites and has a residual body (absent in

[1] Measurements are given in microns throughout.



Figs. 1–4. *I. felis*, from lion. Fig. 5. *I. rivolta*, from tiger. Figs. 6 and 7. *I. rivolta*, from leopard. Figs. 8–10. *I. felis*, from lynx. Figs. 11–13. *I. rivolta*, from jungle cat.

the oocyst). This coccidium resembles *I. felis* in its general structure and dimensions as recorded by Möller (1923), and by others working with cats.

The tigers (*Felis tigris*) harboured two species of coccidia: (*a*) *I. felis* (30 measured) with oocysts 34–45 × 22·2–35, averaging 39·5 × 30·5 with form-index 1:0·57–0·92, average 1:0·76. Spores 19·8–21·9 × 16·5. (*b*) *I. rivolta* (50 measured) with oocysts (Fig. 5) 18–25 × 13–20, averaging 22·1 × 16·5 with form-index 1:0·56–0·87, average 1:0·7. Spores 13·7–17·6 × 10·8–13·2.

The leopard (*Pardus pardus*) harboured oocysts (Figs. 6 and 7) of which 20 measured 20–25·5 × 15–21, averaging 23 × 17·5 with form-index 1:0·69–0·90, average 1:0·8. Spores 13·2–15·2 × 11–12. These are referred to the species *I. rivolta*.

The lynxes (*Lynx lynx*), both from Gandscha and Leningrad, harboured *I. felis* (Figs. 8–10); the oocysts from the Gandscha lynx, it will be observed, were larger:

Gandscha (100 measured) 39·5–50·4 × 30·6–39·6, average 45·5 × 35. Form-index 1:0·69–0·91, average 1:0·76. Spores 23·4–25·2 × 18–21·6.

Leningrad (50 measured) 30–42 × 24·5–33, average 36 × 29. Form-index 1:0·62–0·92, average 1:0·76. Spores 20·9–22 × 17–18·7.

The jungle cat (*Chaus chaus*). Only one animal was examined and found to contain a few oocysts (Figs. 11–13), which from their general appearance were referred to *I. rivolta*. The oocysts measured 23·4–27 × 18–23, with form-index 1:0·76–0·87, average 1:0·76 or 0·87.

SUMMARY.

Two species of coccidia have been found in captive Felidae examined by us in Russia:

(1) *Isospora felis* Wenyon 1923, in lion, tiger and lynx.

(2) *Isospora rivolta* (Grassi 1879), in tiger, leopard and jungle cat.

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ON THE GREGARINE, *LANKESTERIA CULICIS* (ROSS), IN THE MOSQUITO, *AEDES* (*STEGOMYIA*) *ALBOPICTUS* SKUSE.

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(With Plate XXIII.)

Introduction.

In 1895 Ross described *Gregarina culicidis*, a gregarine from a mosquito in India. Subsequently (1906) he renamed it *Lankesteria culicis*. This parasite has been reported since from different parts of the world: by Marchoux, Salimbeni and Simond (1903) from *Aedes argenteus* in South America, by Wenyon in 1911 from the same host in Bagdad, by Stevenson and Wenyon (1915) from West Africa, by Bacot[1] (1916) and Macfie (1917) from West Africa. Lately its range of distribution has been extended up to North China by its discovery in *Aedes koreicus* at Peiping by Feng Lan-Chou. The only author who gives details regarding the life cycle of *L. culicis* is Wenyon (1926).

In September 1931 I came across a batch of larvae of *Aedes albopictus*[2] vigorously growing in a jar full of rain water in our college compound. On examining the guts of these larvae, I found them to be infected with *Lankesteria culicis*.

In studying my preparations, I found that the general plan of the life cycle is as Wenyon described it, but I have observed certain features, such as the occurrence of a well-developed epimerite, polarity in the sporocysts, a different method of association, and other minor features which I consider worth recording.

Observations on the gregarine.

In a fresh preparation of an infected *A. albopictus* gut in saline, gregarines of varying size were seen moving slowly in the field of the microscope. Two types of gregarines could be readily distinguished, one with a very delicate epimerite, the other with a well-developed epimerite. Both types showed the same sort of movements, and the activity became more pronounced in the gametocytes, which I have seen penetrating into the Malpighian tubes as Ross and Wenyon have described. The shape of the body is as described by

[1] Bacot sent some dried leaves of the West African cotton-tree to the Colonial Office. After about three and a half months they were placed in tap water by MacGregor (1915). Both the normal-looking and shrivelled-up eggs of *argenteus* hatched within a few days, and the larvae showed *L. culicis* in them.

[2] My thanks are due to Dr P. Sen, Entomologist to the Public Health Department, Government of Bengal, for kindly identifying the larvae for me.

other workers; the very thin pellicle is marked by delicate longitudinal striations.

The cytoplasm is finely granular, except at the anterior end, where a clear cone-like zone can be distinguished from the rest. Both in smears and sections this clear area takes up a dark, homogeneous stain (Figs. 1–4): therefore I was unable to see the fibres arranged "fan-wise" which Wenyon describes (1911). In my opinion, this well-marked cone-like area should be considered comparable with the protomerite of septate gregarines.

There is much contradiction between the accounts given by different authors of the epimerital structure. According to Wenyon (1911) the epimerite in *Lankesteria culicis* from *Aedes argenteus* is "a vesicle and possibly a suction organ[1] connected with the endoplasm through a pore in the cuticle."..."It evidently functions as an organ of fixation which is used chiefly when the epithelial cell is completely ruptured and the gregarine remains attached to its degenerate remains." From the account of *L. culicis* in *Aedes koreicus* by Lan-Chou (1930) it is evident that the parasites remain attached to the epithelium of the mid-gut by means of a well-developed epimerite (see his Pl. XII, fig. 1). It also appears that they have grown extracellularly from the outset and that their extracellular situation is not due to infected epithelial cells rupturing, as Wenyon suggests, for, if that had been the case, the epithelial lining would not have appeared so uniformly thick throughout the field as is shown in Lan-Chou's figure. According to Marchoux, Salimbeni and Simond (1903), on the other hand, *L. culicis* is a form devoid of epimerite and protomerite, which grows within the cell for most of the time, lying there, pear-shaped and immobile, until it measures 15–30 μ.

I believe that the shape of the epimerite depends on the region of the gut the gregarines inhabit. Those which infest the oesophagus and anterior part of the mid-gut, where the chitinous lining is thin and smooth, are always of intracellular habit and possess very feebly developed epimerites (Figs. 1 and 2). The smallest of these that I have found measures $10 \times 5\,\mu$ and shows a dark zone at the anterior end; at the apex of this zone, or protomerite, there is a minute, hyaline elevation, which establishes contact with the cytoplasm of the host cell. Those gregarines, however, which at an early stage do not succeed in penetrating cells in the anterior region, but pass on toward the posterior part of the mid-gut (where the epithelium is covered by relatively thick ridged chitin), present a very different appearance. Here again the youngest forms I found measured $10 \times 4\,\mu$, but their anterior ends bore a definite epimerite and they were all growing extracellularly (Fig. 3*a*) anchored to the epithelium by the epimerite embedded in the host cell. The epimerite is shaped like an open umbrella connected to the protomerital region by a very short "handle." Occasionally three or four darkly staining granules lie within it. Obviously the nature of the chitinous covering of the epithelium renders the formation of an attaching organ necessary. In *L. culicis*, then, the develop-

[1] He uses the word "suction" with some reserve.

ment of an epimerite seems to be an adaptation to the special circumstances of extracellular life: where a sporozoite is safely lodged within a host cell the epimerite is rudimentary.

It may be argued that the gregarine without epimerite is really a different species from the one having this organ, but, having followed the life history in detail, I am convinced that this is not so. Gametocytes of both forms exhibit no difference in shape or size. The intracellular forms, when they become too large to remain within the host cells, enter the gut lumen, where they then measure 150–194 × 31–41 μ, being broadest just posterior to the hyaline region. The extracellular forms attain the same size, and, when mature, become detached from their epimerites, which are left embedded in the epithelium (Fig. 3*b*). Occasionally I have found individuals 200 μ in length, agreeing better in this respect with Ross's description than with those of Wenyon and the French authors, who give the maximum length as 50 μ. I have never seen forms free in the host's coelomic cavity as described by Marchoux, Salimbeni and Simond.

When a larva is transforming into a pupa, the mature gametocytes actively enter the Malpighian tubes, where they form gametocysts about 50 μ in diameter. The method of association observed by me differs somewhat from that described by Wenyon for *L. culicis*. Judging from my observations the two mature gametocytes associate laterally and not by their anterior ends; for, in most of my preparations, I could not find remains of the epimerite at their point of union as Wenyon shows and as Siedlecki has described in *Lankesteria ascidiae*. Minchin (1916) compares these structures to the secondary nucleus in the protomerite of *Pterocephalus* (*Nina*) described by Léger and Duboscq, which he regards as of a transitory nature and taking no part in the reproductive process. I find that the protomerital region in the gregarine I have studied is always much reduced at the gametocyte stage, and when the larva is about to pupate and the gametocytes to associate there is usually no sign of the protomerite, still less of the epimerite. Therefore, even if they do associate by their anterior ends (which I doubt), it would be surprising to find any remains of structures anterior to the deutomerite. Actually, in two cases where very faint traces of the protomerital region could still be made out, I was able to confirm my own impression that union is lateral (Figs. 5 and 6, where the gametocysts are seen in end-on view).

Prior to nuclear division, and even before the gametocytes have associated (not after encystment, as Wenyon says), there is an increase in the nuclear material, as shown by the greater number of darkly stained bodies fragmented from the karyosome (Fig. 7*a*, *b*, *c*). None of these bodies was seen to travel out of the nucleus, as depicted by Wenyon. The first division of a gametocyte nucleus is shown in Fig. 6. The stages that follow are very similar to those described by Wenyon, except that the general mass of cytoplasm does not seem to fragment in order to provide room for the gametes; nor could I find any trace of anisogamy. The haploid number of chromosomes is three (Fig. 8*a*).

A mature spore measures about $10 \times 6\mu$. In permanent preparations the slightly flattened ends of the sporocysts stain deeply, while the rest remains uncoloured (Fig. 8*c*). In a fresh spore the position of a residual mass (more towards one pole than the other) helps one to distinguish between the poles, which are otherwise identical (Fig. 8*b*). When treated with 1 per cent. methyl green in 1 per cent. acetic acid, this residual mass took a faint greenish stain, but it was completely lost in material fixed for cytological study. Wenyon (1911) does not mention a residuum in the spore, but in his later work (1926) he refers to "a *central*[1] mass of cytoplasm, round which the sporozoites lie."

The parasite is transmitted in a similar manner to that observed by Ross, Wenyon and others. I placed some larvae of *Culex* sp. in the same jar with *Aedes albopictus* to see if they became infected and found that the spores they swallowed passed unaltered with their faeces. This confirms Lan-Chou's observation, for he also failed to infect larvae of *Culex pipiens* with *L. culicis* from *Aedes koreicus*. Perhaps the conclusion that this parasite is confined to mosquitoes of the genus *Aedes* is justified.

SUMMARY.

My observations on *Lankesteria culicis*, as it appears in *Aedes albopictus* in Calcutta, show that the trophozoites are intracellular in the anterior and extracellular in the posterior regions of the mid-gut, with epimerite rudimentary in the former and well developed in the latter condition. Full-grown forms measure 150–200 × 31–41μ. Association is lateral. Sporocysts measure about $10 \times 6\mu$ and are broadly spindle-shaped with slightly flattened ends, with a residual body situated more toward one pole than the other.

Septate eugregarines in general, excepting the Stenophoridae Léger and Duboscq, grow extracellularly with the epimerite attached to the host cell. Therefore, judging from the foregoing description, we are no longer justified in retaining *Lankesteria culicis* among the Haplocyte gregarines (Acephalina) and in the family Diplocystidae Bhatia (1930). The presence of a well-developed epimerite and what I consider to be a rudimentary protomerite, clearly distinguish it from any of the Acephalina. Its place should be among the septate gregarines (Cephalina), and perhaps a new genus may have to be erected to receive it there.

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EXPLANATION OF PLATE XXIII.

All figures are camera lucida drawings either of living specimens or from smears or sections fixed in Bouin-Duboscq-Brasil's fluid (unless otherwise stated) and stained with Heidenhain's iron haematoxylin, except Fig. 7 which is from a smear stained in Delafield's haematoxylin.

Lankesteria culicis (Ross).

Fig. 1. From a transverse section of the anterior portion of the mid-gut, with smooth chitinous lining. Intracellular stage. × 1666.

Fig. 2. The same, showing advanced trophozoites with hyaline, nipple-like structure at extreme anterior end. × 1666.

Fig. 3. From a transverse section of the posterior portion of the mid-gut, with thick, ridged chitinous lining: (*a*) young stage, (*b*) epimerite of an adult left embedded in the host cell. × 1666.

Fig. 4. An adult gregarine (epicyteal striations omitted). From a smear. × 722.

Fig. 5. End-on view of a gametocyst, showing the remnants of the protomerites. From section of pupa fixed in Flemming. × 672.

Fig. 6. The same, but showing the first nuclear division beginning in one of the associates. × 672.

Fig. 7. *a*, *b*, *c*, Showing different stages in the fragmentation of the karyosome. From a smear. × 1800.

Fig. 8. *a*, Spore with zygote nucleus in late anaphase—with three chromosomes at each pole. From section of pupa fixed in Flemming. × 2600.

b, Spore, from a fresh smear, indicating the position of residual mass. × 2600.

c, Spore, from section, showing the deeply stained, flattened poles and four of the sporozoites. × 2600.

[1] This paper was not available to me in Calcutta.

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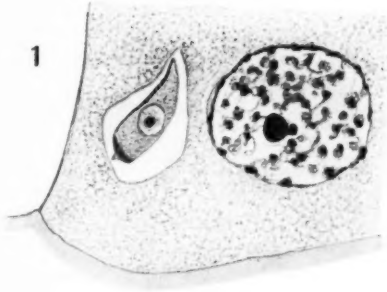

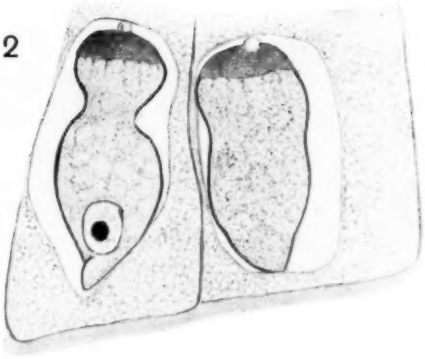

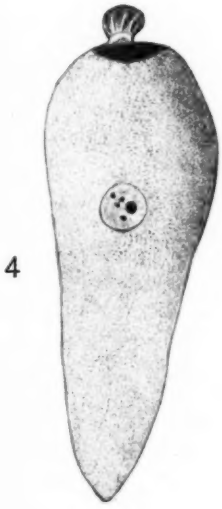

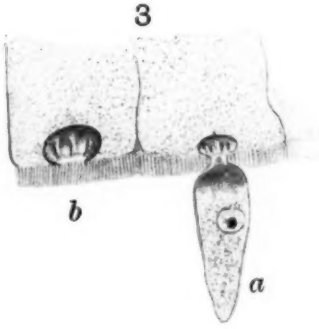

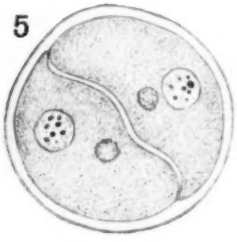

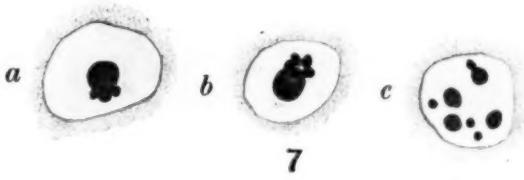

H. Ray del.

THE POSSIBLE RÔLE OF INHERITANCE IN THE QUANTITATIVE CHARACTER OF A COCCIDIAN INFECTION OF THE RAT.

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The writers (1932) have previously reported on the surprisingly great individual variability in susceptibility to infection with *Eimeria miyairii* which they encountered among white rats taken at random. It was learned in the study that young rats of from 65 to 149 g. in weight would eliminate from 14 to 169 million oocysts during immunisation produced by inoculation *per os* with 1500 sporulated oocysts daily over a period of 5 days. The question naturally arises whether different strains of the host exhibit differences in susceptibility similar to those noted in individuals.

The five more or less definite strains of rats employed in the present study are here designated Hi, Lo, Wistar Experimental Laboratory (W.E.L.), Lambert, and Wistar A. The individuals of the Hi strain concerned in the experiment were mostly the F_1 generation obtained by inbreeding within a litter of unknown pedigree (Rats 135–144 in the previous report), using Rat 141 as male and Rats 138, 140, 142 and 144 as females. The Lo strain consisted of F_1 and F_2 progeny from Rats 128 ♂ and 115 ♀, also of unknown pedigree. These rats, as was previously reported, both gave low yields of oocysts. The W.E.L. strain was one not closely inbred, and consisted of the offspring of two females purchased from the Wistar Institute and males of the same strain from our laboratory. The individuals of the Lambert strain were of the fourth to fifth filial generation of a "line inbred" strain, being developed by Dr Lambert of this college. The Wistar A rats used in the experiment represented the 58th and 59th generation of this famous brother-and-sister inbred strain.

The animals were raised in our laboratory, and were definitely known not to have become infected with Coccidia previous to the experimental infections. Their freedom from accidental infection was verified by frequent faecal examination up to the time of the first inoculating dose. All of the 119 rats involved were from 41 to 43 days of age. The growth diet, which was the same for all the rats, consisted of milk and the modified Steenbock ration which has previously been described. Greens were given at intervals. Only the grain mixture was given while the rats were actually on the experiment. The young rats were separated from their mothers promptly on the 30th day

of their lives. The yields of oocysts recorded represent the numbers eliminated by members of the various strains of rats during the process of immunisation. Immunity was produced by inoculation *per os* with 1500 sporulated oocysts of *Eimeria miyairii* daily over a period of 5 days. The technical procedure for collecting and counting the oocysts from individual rats was similar to that previously reported by the writers, except that the counts were made on the basis of 3·6 c.mm. of diluted droppings. The results are summarised briefly in Table I.

Table I. *Numbers of oocysts (in terms of* 10^6*) produced by rats of five strains during immunisation with* Eimeria miyairii.

		Strains of rats					
	Constants	Lambert	Lo	Hi	W.E.L.	Wistar A	Totals
Males	N	8	14	20	8	9	59
	M_x	36·1	94·9	194·4	64·1	175·7	128·8
Females	N	7	14	21	10	8	60
	M_x	31·9	68·6	187·3	89·2	148·4	119·9
Totals	N	15	28	41	18	17	119
	M_x	34·1 ± 3·9	81·8 ± 5·5	190·8 ± 7·8	78·1 ± 5·8	162·8 ± 7·2	124·3
	Σ_x	512	2289	7822	1405	2768	
	R_x	16–90	47–181	60–366	27–160	77–239	16–366
	$C.V._x$	60·1 %	52·8 %	39·2 %	46·8 %	26·8 %	

N = no. of rats.
M_x = mean no. of oocysts.
Σ_x = sum of oocysts.
R_x = range in no. of oocysts.
$C.V._x$ = coefficient of variation for nos. of oocysts.

The five strains of rats were found to exhibit vast differences in susceptibility to coccidiosis, as measured by the number of oocysts eliminated during immunisation after standard doses of the micro-organism. The Lambert strain showed not only the lowest mean yield, but also the greatest variability in the susceptibility of its members, as indicated by the coefficients of variation. Two other moderately low yielding strains, the Lo and the W.E.L., likewise showed considerable variability. The high-yielding strains, the Wistar A and the Hi, showed much less variability, with the Wistar A the lower of the two. Low variability, of course, would be expected in a highly inbred strain if heredity governs susceptibility to any extent.

The data obtained by the counts were subjected to statistical analysis by Brandt's generalisation of Fisher's method for the analysis of variance. The writers have had the personal assistance of Dr Brandt in applying the method. The analysis (see Appendix) gave a z value of $+1{\cdot}7645$ for the comparison of the variance between strains, exclusive of sex, with the experimental error—an exceedingly significant figure, since 0·6 is highly significant. The differences between strains, then, could hardly be attributed to chance alone. The conclusion which is naturally suggested is that the rats reacted differently to the presence of the micro-organism in their digestive tracts because of inherent differences.

The writers stated in their previous report that the males in a series of random rats exhibited a significantly greater susceptibility to the infection than the females. It was explained, however, that in most cases the males and females were drawn from different litters (curiously enough), and that the results could be accepted only with reservation. In the present series whole litters were employed; therefore, there was no selection of sexes within a strain. When the data in the present series were subjected to analysis by the Fisher-Brandt method (see Appendix) so as to test the difference between males and females, a z value of $-0{\cdot}0728$, a non-significant figure, was obtained for the variance due to comparison of sex, exclusive of strain, with the experimental error. Therefore, the differences between sex were no greater than might be accounted for by chance alone. The mean oocyst yield from the males was 128,814,000; from the females, 119,933,000.

Discussion.

The results of the experiment appear to be clear in the matter of the existence of groups of related rats which behave differently in their reaction to the *Eimeria miyairii* infection. That is to say, an investigator might expect the quantitative character of the infection in a population of rats with one hereditary make-up to present an altogether different picture from that exhibited in a comparable series of rats of differing heredity. The two series of hosts to be comparable, of course, must have been separated from their mothers at the same age, must have received the same diet in the same amounts after weaning, and must have been infected at the same age and with the same size dose of the same strain of parasites. The writers believe that their animals fulfilled all these conditions; and, in addition, the rats were kept in the same room and cared for by the same persons. It was neither possible nor practicable for us to test all the rats at the same time, but it was often noted that the strain differences were manifested when groups of individuals belonging to two differing strains were tested within a day or two of each other.

The writers are not prepared to make any claim that the progeny of the individuals tested will react to the infection exactly as their progenitors whose host potentialities are here reported. It would probably depend entirely upon how the matings were made whether or not the present characteristics of the strains would be preserved. It is to be expected that the progeny of the Wistar A strain will continue to exhibit high average susceptibility and low variability, providing the close inbreeding is continued. Outbred lines of the W.E.L. strain should continue to manifest a rather low average susceptibility and a high variability. It is impossible to predict the future course of the Lo strain and the Hi strain with continued inbreeding, because they are probably highly heterogeneous. In the case of the Lo strain, low-yielding individuals are being mated; in the case of the Hi strain, high-yielding individuals.

Whether the type of infection can be fixed probably depends to some extent upon the number of matings from which selections are made, and upon whether the important factors which govern susceptibility and resistance are dominant or recessive. The writers recently secured from Dr Lambert another pair of rats very closely related to the parents of the 15 rats comprising the two litters listed in Table I. These rats had ten offspring which, in a test just completed, showed the highest yields of oocysts of any litter we have yet tested. Evidently this strain is still highly heterogeneous in its susceptibility to coccidiosis; hence, no prediction regarding its future trend can be made.

Are the facts herewith submitted applicable to other kinds of animals, such as cattle, sheep, pigs, rabbits and chickens? There is little information available with which to answer the question. The situation in regard to these forms urgently requires further investigation. It might be suggested that there is a prevalent belief that White Leghorn and Rhode Island Red fowls are more susceptible to coccidiosis than Plymouth Rocks. The writers, however, have made no observations on the quantitative character of coccidian infections in the domestic fowl.

Can inbred strains of animals relatively resistant to coccidiosis be developed? This again is a question to which no answer can be given at present, for the writers have accumulated very little information regarding it; but their meagre observations seem to indicate that outbreeding provides a population possessing a low mean, though highly variable, susceptibility. The series of 48 rats employed in the previous study was entirely outbred.

Conclusions.

1. There exist strains of white rats which, upon experimental tests, exhibit diverse susceptibilities to infection with *E. miyairii*, as judged by the numbers of oocysts eliminated during the process of immunisation.

2. Since all the rats on the test were reared and maintained under laboratory conditions which were as nearly uniform as possible, it is to be concluded that the likely explanation of the variance is to be found in the heredity of the hosts.

3. The measurements of susceptibilities of the two sexes were found not to be significantly different when the individuals tested were comparable in as many respects other than sex as it was possible to make them.

Acknowledgments.

This investigation was supported by a grant from the Rockefeller Fluid Research Fund at Iowa State College.

The authors wish also to express their gratitude to Dr W. V. Lambert for giving us rats of an inbred strain, and to Dr A. E. Brandt for help with statistical methods.

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APPENDIX.

Details of analysis of variance.

Source of variation	D./F.	Sum of squares of deviation from mean	Mean squares	1/2 nat. log.
(1) Sex	1	2,594·6	2,594·6	3·9306
(2) Strains	4	417,565·6	104,391·4	5·7779
(3) Interaction	4	8,805·3	—	—
(4) Experimental error	109	332,962·9	3,054·7	4·0134
(5) Total	118	761,927·6	—	—

z for sex $= 3{\cdot}9306 - 4{\cdot}0134 = -0{\cdot}0828.$

z for strain $= 5{\cdot}7779 - 4{\cdot}0134 = +1{\cdot}7645.$

Note. The reason why the items in the "Sum of Squares" column are not additive is that there are unequal numbers in the different categories of the classification. See Brandt, A. E., *J. Amer. Statistical Assoc.*, June, 1933.

(*MS. received for publication* 3. I. 1933.—Ed.)

SPOROGONY IN *SELENIDIUM MESNILI* BRASIL, A SPOROZOAN PARASITE OF *MYXICOLA INFUNDIBULUM* MONT.[1]

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(With Plates XXIV–XXVII, containing Figs. 1–32, and Figs. I–III in the Text.)

I. Introduction.

In 1930 Ray published a valuable paper on the genus *Selenidium* Giard, in which he described sporogony in *S. spionis* Koll. and *S. foliatum* Ray. He did not observe schizogony in either of these two, but he confirmed Brasil's account of intracellular schizogony in *S. caulleryi* Brasil from *Protula tubularia* Mont. and recorded it for the first time in *S. mesnili* Brasil from *Myxicola infundibulum* Mont. After seeing association of gametocytes in both these species he was unable to continue his observations, so I have taken up the work where he left it, choosing *S. mesnili* rather than *S. caulleryi*, since it is more easily obtainable. My search for gamete and sporozoite formation has been successful, but I have not been able to make the sporozoites hatch, or in studying sections of *Myxicola* have I seen sporozoites entering the epithelial cells of the gut.

II. Material and methods.

Since *Myxicola infundibulum* lives at the low-tide mark it can be obtained only at spring tides and then not in midsummer. The material used was collected in August from Salstone when I was at Plymouth. The worms were kept in shallow dishes of sea water which was changed daily, and there they remained quite healthy for a fortnight, though towards the end of this time their faeces became scanty.

The faeces were removed with a pipette every morning and at intervals during the day, and samples were examined as smears. The faeces always contained abundant *Selenidium mesnili*, at first in gametocysts, later, as the worms became defaunated, with trophozoites. As a rule each faecal pellet contained only one stage of the life cycle. I attach great importance to the study of living material, and so far as possible have checked my observations based on sections and smears by drawings made from life. Unfortunately, though material was often kept in a moist chamber, it never developed far, probably on account of bad aeration.

[1] Thesis approved for the degree of Master of Science in the University of London.

The smears were fixed with Brasil's modification of Bouin-Duboscq's fluid, and stained with Erlich's or Delafield's haematoxylin (sometimes followed by orange G); both fixative and stain were used overnight. For sections the faeces were fixed in Bouin-Duboscq, embedded, cut and stained in Heidenhain's haematoxylin and counterstained in chromotrop 2R.

III. SPOROGONY IN *SELENIDIUM MESNILI* BRASIL.

(*a*) *Gamete formation.*

(Pl. Figs. 1–11[1].)

Ray (1930) described the trophozoites (Fig. I[1]) in the gut wall of *Myxicola infundibulum*, and followed the processes of intracellular schizogony. He also stated that before association the trophozoites drop out of their host cells and

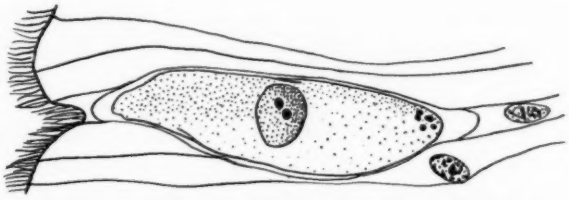

Fig. I. Trophozoite in an epithelial cell of the gut of *Myxicola infundibulum* (× 650). (From one of Dr Ray's sections.)

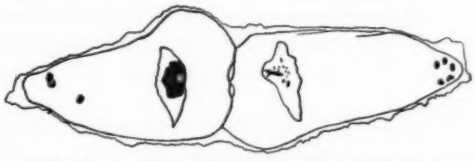

Fig. II. Gametocytes in the lumen of the gut, associating by their posterior ends (× 650). (From one of Dr Ray's sections.)

associate by their posterior ends (Fig. II); but from examining his sections of the gut I have come to the conclusion that the heavily infected patches of gut wall break down, liberating a mass of gametocytes and degenerate epithelium into the lumen of the gut. Before or after association, the nuclei of the gametocytes elongate transversely. The gametocytes secrete round themselves a soft, gelatinous wall, which conforms to the changing shape of the gametocytes within (Fig. 1). The gametocytes become shorter and thicker, rolling round each other. The internal diameter of the cysts varies from 21 μ to 76 μ, the average being 50 μ. The cysts swell in sea water and disintegrate in a few days. Occasionally three gametocytes come together and encyst; their fate is not known.

The nuclei of the gametocytes discard their karyosomes (Fig. 1) and the

[1] Plate figures bear *arabic* numbers, text figures bear *roman* numbers.

chromatin which was condensed round these becomes scattered on a reticulum throughout the nucleus. The terminal chromatic dots in the cytoplasm become somewhat scattered, but apparently persist until the sporozoites are formed; it seems that they do not give rise to the new terminal dots of the ultimate sporozoites any more than they do to those of the merozoites in schizogony (see Ray, 1930).

The first nuclear division is mitotic, with a definite spindle, though at this stage no centrosomes or asters are visible (Fig. 3). The daughter nuclei are small and stain homogeneously and lightly. Subsequent mitotic divisions are scattered throughout the gametocytes (Fig. 4); I have seen no trace of multipolar fission such as Ray thinks possible in the schizogony of this species, or of intranuclear spindles such as Caullery and Mesnil (1899) and Ray (1930) describe in the gametogony of *Selenidium caulleryi*. The nuclei at a somewhat later stage show the chromatin separating into a central mass and a peripheral layer connected therewith by radial threads (Fig. 5). The nuclei go to the periphery of the gametocytes and divide again; a centrosome appears outside the nuclear membrane towards the periphery of the gametocyte (Fig. 6, *a*) and divides, the daughter centrosomes moving to the opposite poles of the nucleus (Figs. 6, *c* and 7); either before or after this the chromatin forms a fine reticulum studded with granules (Fig. 6, *c*, *d*). The whole spindle stains darkly so that even when the spindle is fully formed (Fig. 6, *e*) no chromosomes can be distinguished, but in late metaphase some three to five can be seen (Fig. 8). The daughter nuclei become vesicular, each with a large central mass of chromatin containing a small karyosome and suspended by fine threads from the nuclear membrane (Fig. 9: this figure is drawn from a tangential section of a cyst so that the nuclei appear to be scattered throughout a gametocyte).

The cytoplasm now begins to shrink away from the gametocyst in preparation for cleavage.

Cytoplasmic cleavage begins at the periphery and extends gradually inwards, dividing the gametocytes completely and somewhat irregularly, without leaving any residual mass (Fig. 10); a few gametes therefore contain the terminal dots of the parent gametocytes. A darkly-staining substance is deposited in the clefts between the gametes and between the gametes and the cyst. Usually one gametocyte divides somewhat before the other, but there is complete isogamy. The average diameter of a gamete is 7μ. Meanwhile the karyosomes of the gametes disappear (Fig. 10, where only two nuclei show them), the nuclear membrane becomes less definite and the suspending threads disappear. The central chromatin mass now sends out chromatin threads (Fig. 11, *a* and *b*) until the nucleus is filled with a coarse reticulum studded with granules of chromatin (Fig. 11, *c*).

(*b*) *Fusion of gametes.*

(Pl. Figs. 12–16.)

The gametes now begin to fuse in pairs. The first to unite are those lying where the two gametocytes are in contact (Fig. 12); the remoter gametes at first stay in their original positions, but shrink a little from each other, as can be seen from the gaps between them and the dark intergametic substance. The gametes as they come together retain the irregular shape impressed on them by mutual contact (Fig. 13). The zygote soon becomes spherical and secretes round itself a thin, smooth, darkly-staining cyst, which, unlike the gametocyst, shows no sign of swelling and disintegrating in sea water (Fig. 14). The average diameter of a sporoblast is 10·5–12·5 μ; about 100 sporoblasts are formed.

The gamete nuclei contain coarse chromatin granules at the periphery suspended on an achromatic reticulum (Fig. 14, *a*). The two nuclei now begin to fuse (Figs. 14, *b* and 15) and the chromatin eventually forms a compact ball suspended by fine threads in the centre of the zygote nucleus (Fig. 16, *a*).

(*c*) *The zygote nucleus.*

(Pl. Figs. 16–21.)

The ball of chromatin swells, becomes less homogeneous (Fig. 16, *b*) and develops into a reticulum (Fig. 16, *c*) and then into a tangled spireme studded with chromatin granules (Fig. 17). This spireme becomes shorter and thicker, contracting towards one side of the nucleus (Figs. 18 and 19). It then casts out wavy threads which look remarkably like paired chromosomes (Fig. 20), which disintegrate into granules again (Fig. 21).

IV. NUCLEAR DIVISION IN THE SPOROBLAST.

(Pl. Figs. 22–28.)

Two centrosomes appear close to the nuclear membrane, apparently inside it (Fig. 22), and move to opposite poles of the nucleus (Fig. 23). The chromatin expands to fill the nucleus, and becomes at first coarsely, then more finely granular (Figs. 22 and 23, *a* and *b*). The resemblance between the nuclear figures in Fig. 6, *e*, Fig. 23, *b*, and Ray's Plate XXXVI, figs. 4*c* and 4*d*, is striking, and suggests that nowhere in this species are chromosomes visible in the earlier stages of nuclear division. They are visible in the late anaphase as short, thick, bent rods, but I was not able to count them accurately since they lay close together and on top of each other. Only one gametocyst in my preparations showed this stage (Fig. 24). As the chromosomes pass to opposite poles of the spindle a thread is left between the sister nuclei which gives to the whole a somewhat dumbbell-shaped appearance (Fig. 24, *b*).

The two nuclei of the sporoblast show no definite nuclear membrane, but the chromatin spreads out as a layer of coarse granules on the periphery of a

fairly darkly-staining central mass. This stage evidently lasts but a short time, for it is very uncommon in my preparations (Fig. 25, *a*). Two centrosomes soon appear (Fig. 25, *b*) and pass to opposite poles of the nucleus while the chromatin becomes scattered as a fine dust between them (Fig. 26). In anaphase the nuclei again present a dumbbell-shaped figure, the chromatin in which is condensed into uniformly black masses (Fig. 27). It spreads out, becoming granular and reconstitutes four resting nuclei, each of them having a peripheral network, with granules of chromatin where the threads join, but none in the centre (Fig. 28, *b*).

(*d*) *Sporozoite formation.*

(Pl. Figs. 29–32.)

The four nuclei move to one side of the spherical sporocyst. The chromatin in each rearranges itself as a layer of peripheral granules to which are attached threads supporting a central mass (Fig. 29, *a*). Cytoplasmic cleavage begins at the nuclear end of the sporoblast and extends gradually to the opposite

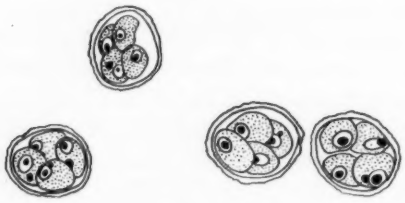

Fig. III. Sporozoites inside sporocysts after the disintegration of the gametocyst; stained in Lugol's iodine (× 960).

pole (Fig. 29, *b*). A darkly-staining granule then emerges from the nucleus into a clearer area of protoplasm (Fig. 30); it moves away from the nucleus, grows and divides by constriction (Fig. 31) until there are three to five in each sporozoite (Fig. 32). Meanwhile the sporozoites become narrower, longer and more curved, so that no sporozoite can be entirely seen in any one focus, but Fig. 32 and Fig. III show clearly that four are present, a number that is not uncommon in the Coccidia, but which forms a marked contrast to that of the majority of the gregarines, which is typically eight.

V. Note on reduction division.

I had hoped that my investigation might shed some light on the question of reduction division in *Selenidium*. Unfortunately, the gametocysts of *S. mesnili* are somewhat impervious to fixatives and the sporocysts are extremely so, and probably it was the consequent slow fixation that caused clumping of the chromatin during nuclear division so that the number of chromosomes could not be accurately determined at any stage. The number is certainly small and may be uneven, about three to five showing in Figs. 3, 4

and 8. Unless the number of chromosomes has been accurately determined, reduction division cannot be positively affirmed at any stage; but the close resemblance of certain nuclear figures in this species to those that Jameson (1920) draws of the eugregarine *Diplocystis schneideri* Kunst., makes it not improbable that similar changes occur in both gregarines. Jameson was able to show that reduction occurred in *D. schneideri*, not before but after gamete formation. That the same is true of *Selenidium mesnili* seems likely from the apparent synapsis (Fig. 20) suggesting the phase which in the higher animals immediately precedes reduction.

VI. CONCLUSIONS.

Before sporogony the infected patches of the gut wall break down, liberating the gametocytes into the lumen of the gut where they associate. The gametocytes encyst in pairs and are thrown out of the gut into sea water where they continue their development. The gametocyte nuclei divide repeatedly by mitosis. Numerous gametes are formed. There is no residual mass. Isogamy is complete. The sporocysts are smooth and spherical. Reduction probably occurs immediately after the fusion of gametes. Four sporozoites are formed in each sporocyst. The nucleus of each sporozoite gives rise to one terminal dot, which in turn divides to form others.

I have great pleasure in thanking Prof. Mackinnon, at whose suggestion this work was undertaken and under whose direction it was carried out; and Dr Ray, who gave me technical advice and allowed me to examine his preparations.

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EXPLANATION OF PLATES XXIV–XXVII.

Illustrating *Selenidium mesnili* Brasil.

All figures are drawn with the aid of a *camera lucida* from sections of material fixed in alcoholic Bouin, and stained with Heidenhain's haematoxylin, counterstained with chromotrop 2R.

PLATE XXIV.

Figs. 1–4, magnification × 1560.

Fig. 1. Association. Note the transverse elongation of the nuclei, the discharge of the chromatic granule from the karyosome of one of them, and the terminal position of the extranuclear, chromatic dots.

Fig. 2. Association. Note the shortening of the gametocytes, and the discharge of the previously intrakaryosomic granule from the nucleus.

Fig. 3. The first nuclear division of one of the gametocytes.

Fig. 4. Later nuclear division in the gametocytes. Note the scattered nuclei and the position of the extranuclear chromatic dots; also the spherical shape of the gametocyst.

PLATE XXV.

Figs. 5–10, magnification × 1560.

Fig. 5. Gametocytes containing a few resting nuclei.

Fig. 6. Prophase of nuclear division in the gametocytes; **a**, appearance of a centrosome; **b**, disintegration of the karyosome; **c**, division of the centrosome; **d**, separation of the daughter centrosomes; **e**, daughter centrosomes at the opposite poles of the nucleus. Note the peripheral position of the nuclei.

Fig. 7. Prophase of nuclear division in the gametocytes, showing the division of the centrosome before the disintegration of the karyosome.

Fig. 8. Anaphase of the nuclear division in the gametocytes. Drawn from a section stained by Mève's method.

Fig. 9. Gametocytes containing numerous resting nuclei. Drawn from a tangential section of a gametocyst.

Fig. 10. An early stage in the formation of gametes. Clefts beginning at the periphery of the cytoplasm have not in all cases reached the centre of the gametocyte; **a**, two nuclei still retaining the intrakaryosomic chromatic granules.

PLATE XXVI.

Figs. 11 and 12, magnification × 1560; Figs. 13, 14, 16, magnification × 3120.

Fig. 11. A later stage in the formation of gametes, showing changes of structure in their nuclei; **a**, the discharge of chromatic threads from the karyosome; **b**, further discharge of threads, and the beginning of a reticulum; **c**, a nucleus containing a reticulum.

Fig. 12. The fusion of gametes. Note the binucleate zygotes in the middle of the gametocyst, and the shrinkage of the remoter gametes.

Fig. 13. Two gametes fusing.

Fig. 14. Binucleate zygotes within smooth, spherical sporocysts; **a**, one nucleus lying above the other but not yet fused with it; **b**, the beginning of nuclear fusion.

Fig. 16. Uninucleate zygotes (sporoblasts); **a**, the chromatin of the nucleus in a compact, central ball; **b**, the ball of chromatin unravelling; **c**, the chromatin in the form of a reticulum.

PLATE XXVII.

Figs. 15, 17–32, magnification × 2500.

Fig. 15. A zygote in a late stage of the fusion of nuclei, the chromatin of which is condensing into compact, central masses.

Fig. 17. A sporoblast containing a large nucleus whose chromatin is in the form of a spireme.

Fig. 18. A sporoblast the chromatin of whose nucleus is beginning to contract.

Fig. 19. A sporoblast whose nucleus shows extreme contraction of the chromatin to form a short, thick thread at one side of the nucleus.

Fig. 20. A sporoblast whose nucleus shows synapsis.

Fig. 21. A sporoblast whose nucleus is elongating, in preparation for division.

Fig. 22. A sporoblast showing the nucleus in the early prophase of the first nuclear division, with the chromatin in a confused reticulum.

Fig. 23. Sporoblasts in later stages of prophase; **a**, the reticulum breaking down; **b**, the chromatin scattered in the form of a fine dust, containing a few larger granules.

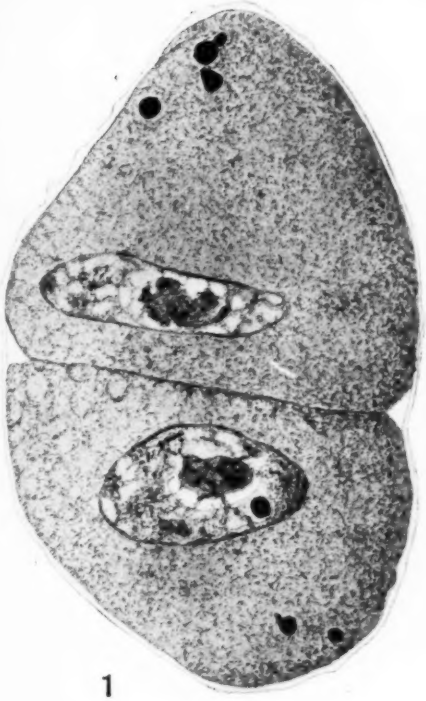
Fig. 24. Four sporoblasts from one gametocyst; **a**, metaphase of the first nuclear division; **b**, anaphase.

Fig. 25. Sporoblasts; **a**, two resting nuclei; **b**, one resting nucleus and one in prophase.

Fig. 26. A sporoblast showing one nucleus in early, the other in later, prophase.

Fig. 27. A sporoblast showing two nuclei in anaphase.

Fig. 28. Two sporoblasts from the same gametocyst; **a**, an early stage in the reconstruction of the four daughter nuclei; **b**, four resting nuclei in the centre of the sporoblast.



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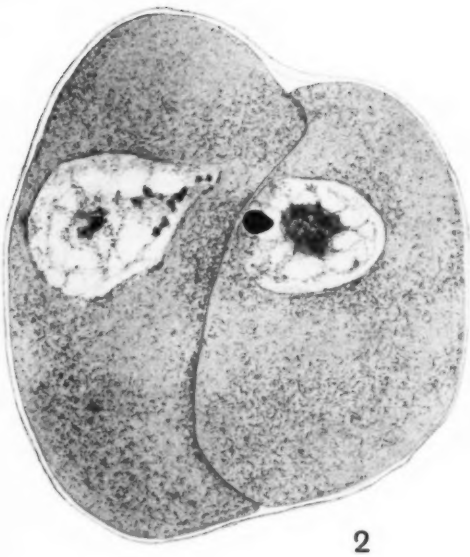

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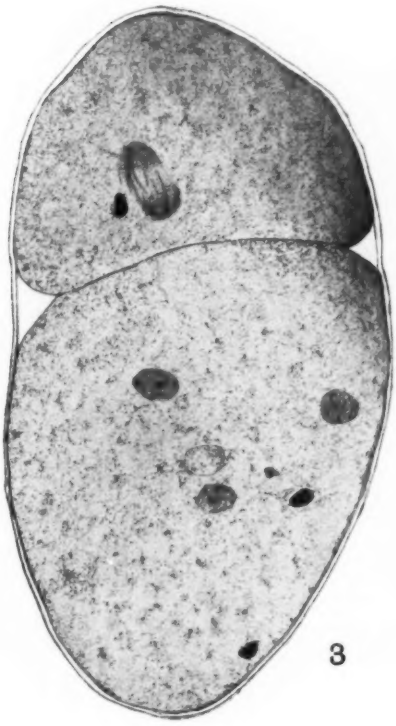

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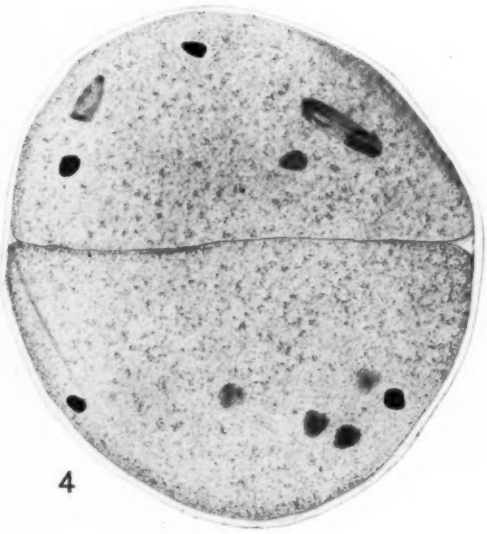

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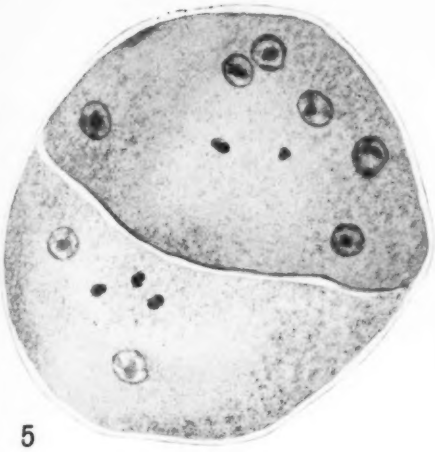

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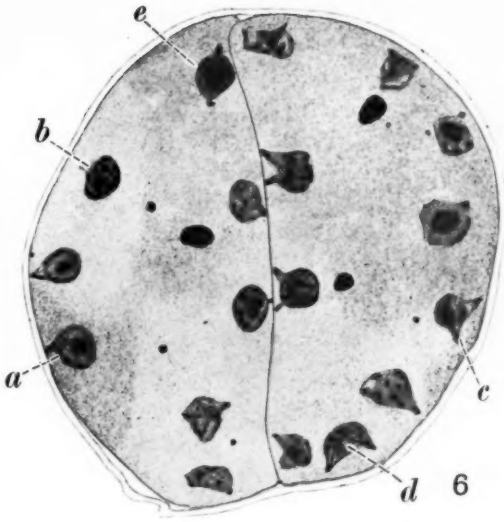

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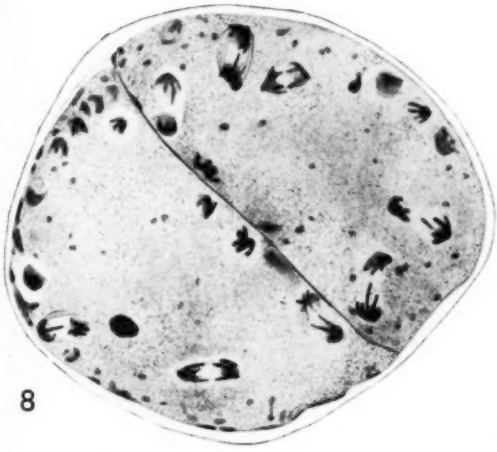

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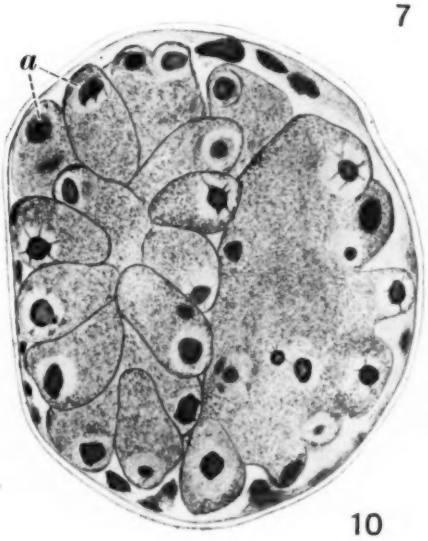

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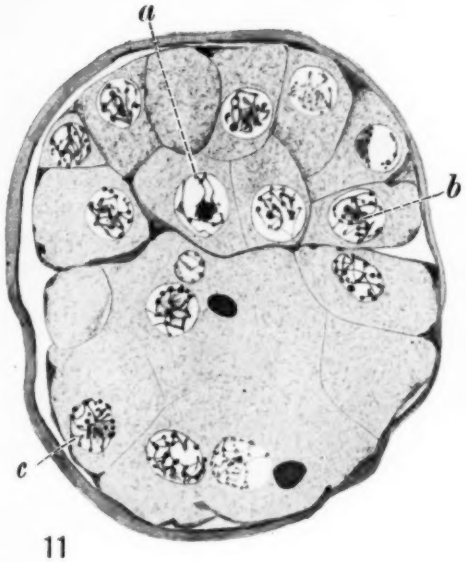

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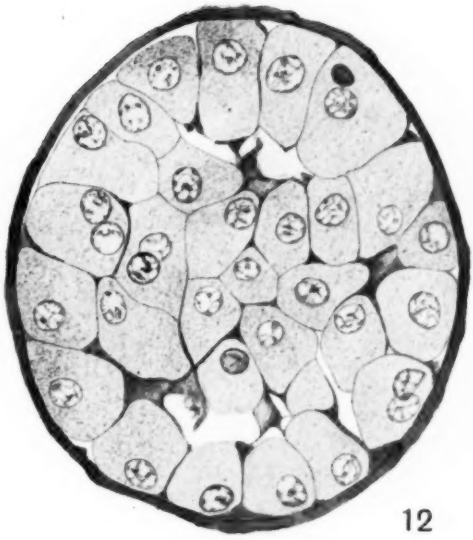

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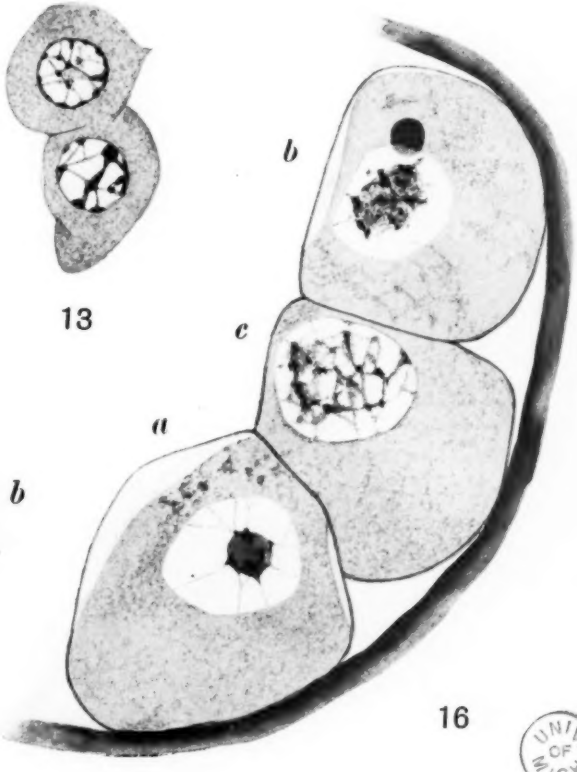

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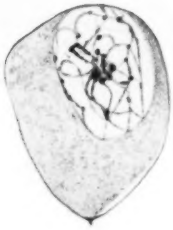

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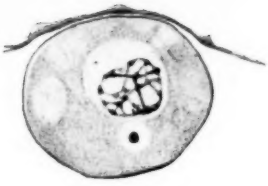

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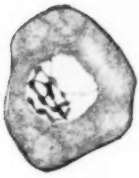

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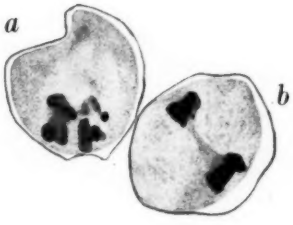

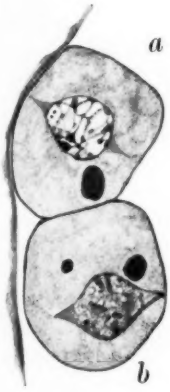

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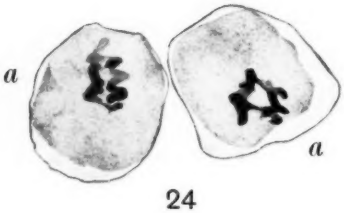

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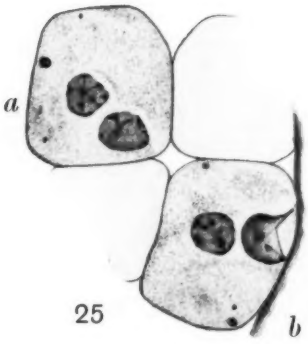

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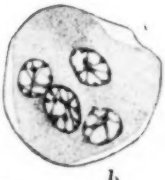

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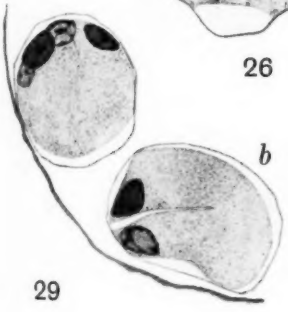

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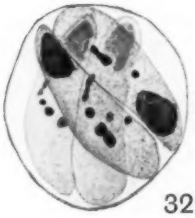

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Fig. 29. Sporoblasts; **a**, four resting nuclei lying at one end of the sporocyst; **b**, the beginning of cytoplasmic cleavage at this end of the sporoblast, to form sporozoites (only two nuclei are shown).

Fig. 30. Two of the four sporozoites in a sporocyst. Note the origin of a chromatic dot from the nucleus of one of them.

Fig. 31. Sporozoites with one extranuclear chromatic dot apiece (only three of the four sporozoites are shown).

Fig. 32. Four sporozoites in a sporocyst. Note the dividing chromatic dots, and their movement away from the nuclei.

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CORRECTION.

This volume, p. 252, line 16, for "*Jajonetta* n.g." read "*Ceratotrema* n.g."